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CHANGES IN THE CENTRAL INCISORS OF HYPOPHYSECTOMIZED FEMALE RATS AFTER DIFFERENT POSTOPERATIVE PERIODS

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EXTENSIVE studies on the relation of the pituitary gland to skeletal growth have led naturally to investigations of the relation to the development of the teeth. In the rat, hypophysectomy after 1 month of age results in immediate cessation of skeletal growth, including regressive changes in the cartilaginous epiphysial plate, disappearance of bone trabeculae, increase of the fat content in the marrow of the epiphysis and the diaphysis and "sealing off" of the epiphyseal cartilage by bone. For a recent and complete account of the changes in the osseous system of the rat at different intervals after hypophysectomy, reference may be made to Becks, Simpson and Evans.[1] A brief review of earlier work on the subject is also contained in that paper.

The changes in the incisor of the white rat following hypophysectomy were first observed histologically and roentgenographically by Schour and Van Dyke.[2] These authors made postmortem studies of 23 completely hypophysectomized rats which had been 36 to 64 days old at the time of operation and which were dead 2 to 15 months after operation. These animals were classified into three groups according to the roentgenographic and histologic observations:

Group I comprised 8 animals which lived from 2 to 6 months after the operation and which presented characteristic early changes: The

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1. Becks, H.; Simpson, M. E., and Evans, H. M: Anat. Rec. **92**:109, 1945.
2. Schour, I., and Van Dyke, H. B.: Am. J. Anat. **50**:397, 1932.

contour of the labial surface of the incisor was composed of two segments ". . . an anterior larger segment of a small circle and a posterior smaller and flatter segment of a larger circle." A sharp indentation of enamel in the region of the junction of the apical and the middle third, and enamel globules projecting on the surface, were seen in the incisors of this group. Folding of enamel epithelium was a feature, and hypoplasia of enamel occurred sometimes to the extent that in spots no enamel was formed; at these places the enamel epithelium met the dentin surface. This folding was also observed in the roentgenograms.

Group II consisted of 8 animals which lived 6 to 10 months after the operation and showed intermediate changes in the apical portion of the incisor: Irregular contour of the labial surface at the apical end and partial absence of enamel formation were observed. The enamel matrix showed deep slits with epithelial and connective tissue ingrowth and partial degeneration of the enamel epithelium. The dentin had many tubular spaces, with complete perforation in some places. The enamel and the dentin on the labial surface were folded in the most posterior portion of the incisor.

Group III comprised 7 animals that lived from 10 to 15 months after the operation and presented advanced changes: Here calcification of the enamel in the apical region was incomplete and its labial outline irregular. The ingrowths were more pronounced, and multiple foldings (with three or four deep folds and a few minor folds) had formed, accompanied by severe displacement of Hertwig's sheath. The enamel epithelium was severely degenerated, and with the exception of a narrow slit the pulp was almost entirely obliterated. This condition was described as a result of continued dentin formation and lack of tooth eruption.

These authors summarized the findings of Groups II and III as follows: "The radiograph of the incisor of the hypophysectomized rat that lived a given period after the operation gives a constant pathognomonic picture". It must be noted that the conclusions of Schour and Van Dyke for groups II and III were based on 15 rats. These included 10 rats which died during the course of their study.

Our purpose in the present paper is to report a restudy of the question of the effect of hypophysectomy on tooth structures. This study was made with a larger number of animals, representing a greater range of time after the operation.

EXPERIMENTAL MATERIAL

One hundred and forty-one female rats [3] of the Long-Evans strain were hypophysectomized [4] when they were between 26 and 28 days of age. Only animals

3. A preliminary report on part of these animals is contained in the descriptions of hypophysectomized controls to experiments on the effects of hormone

in which the operation was complete have been included in this report. The completeness of operation was judged by the following criteria:

1. Growth curve: Little if any gain is noted in body weight and length after operation.
2. Muscular tone: After long postoperative periods the muscle tone is poor.
3. Condition of hair: The hair is soft, fluffy and long. The coarse guard hairs observed in normal animals are lacking.
4. Estrous cycles: There is complete suppression, and the vagina is closed.
5. At autopsy: The thyroid gland, the cortex of the adrenal gland and the gonads are atrophic.
6. The sella turcica (examined with dissecting binoculars) shows no remains of the gland.
7. Serial sections of any suspicious fragments of tissue in the sella turcica reveal no hypophysial remnants.

The rats were killed from 6 to 664 days after operation (table 1) and the skulls roentgenographed. The skulls of half of the older animals, those with a postoperative period between 316 and 664 days, were prepared for histologic studies. The material was fixed in neutral solution of formaldehyde U. S. P. (1:10), decalcified in 5 per cent nitric acid, embedded in pyroxylin, and the sections stained with hematoxylin-eosin.

RESULTS

Roentgenographic Aspect.—The study of roentgenograms of the skull disclosed changes in the incisors which increased in severity with increase in length of the postoperative periods. During the shorter periods, 6 to 12 days after operation, no abnormal changes could be detected (fig. 1*A*). After 14 days, thickening of the dentin on the labial side of the upper incisor (fig. 1*B*) was observed; this represents the earliest effect of hypophysectomy on the incisors. It was present in all animals 14 or more days after the operation and is designated as stage 1. At 87 days [5] after hypophysectomy thickening of the lingual dentin became definite (fig. 1C); it was present in all animals after this postoperative interval and constitutes stage 2. After 100 days an abrupt increase in the curvature of the apical region on the labial side was seen; this will hereafter be described as a distortion of the apical curvature (fig. 1*D*). This change characterizes stage 3. Definite folding of the apical end of the upper incisor was seen for the first time 144 days after

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therapy on the teeth of hypophysectomized rats (Collins, D. A.: The Effect of Experimental and Endocrine Disturbances on Dental Structures of Dogs and Rats, Thesis in partial fulfilment of the requirements for the Degree of Master of Science in Dentistry, University of California, 1944).

4. The method of operation was a modified Smith parapharyngeal approach (Smith, P. E.: J. A. M. A. **88**:158, 1927; Am. J. Anat. **45**:205, 1930).

5. Because of the rather long interval of time between the postoperative periods of forty-three and eighty-seven days for which no observations are available, it must be considered possible that a lingual thickening may occur before eighty-seven days.

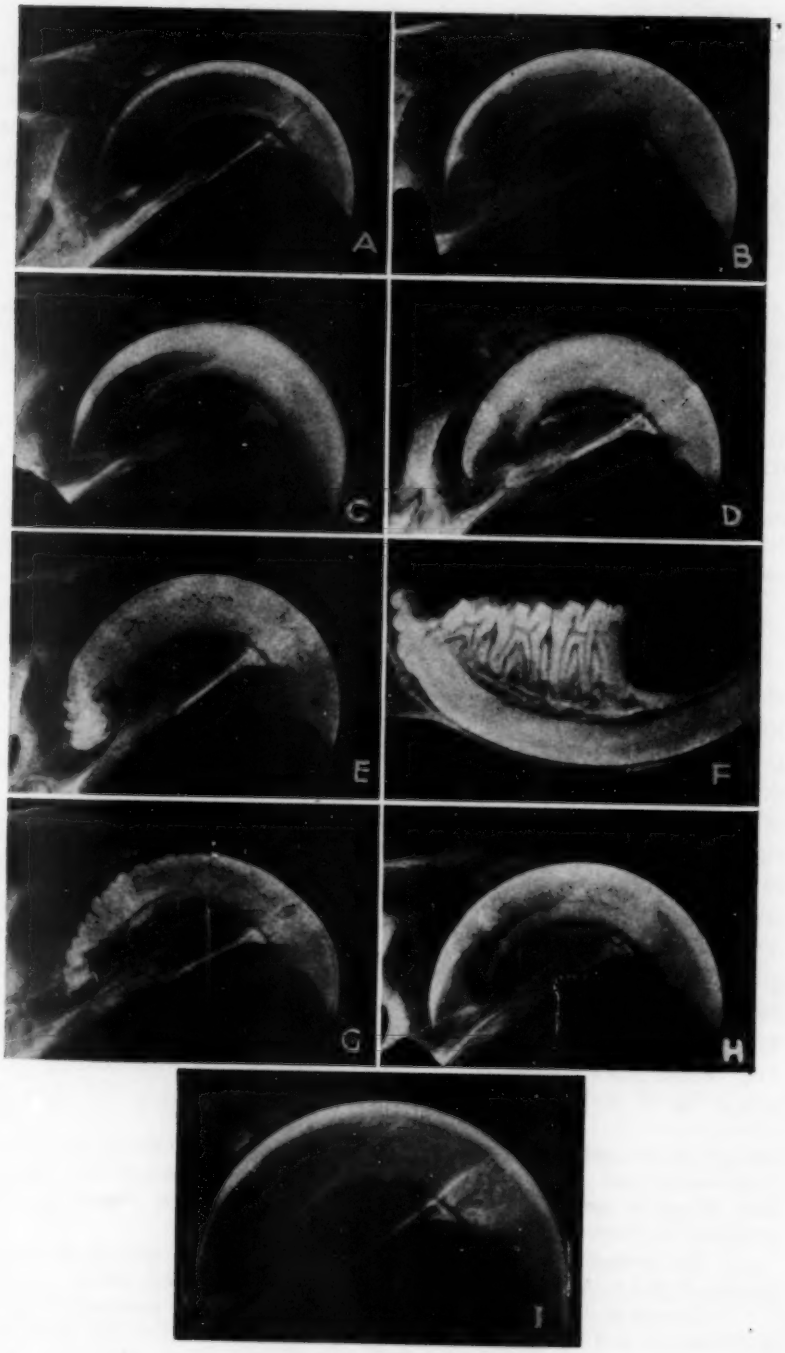

Figure 1

(See legend on opposite page)

the operation (fig. 1E) and is called stage 4. Folding of the apical end of the lower incisor (fig. 1*F*) was not observed before 205 days and even then occurred only occasionally; this change characterizes stage 5. Folding was never observed in the lower incisor only. Thus the roentgenographic changes fall into five definite stages. While the first stage occurred in all animals 14 days or more after operation and the second in all animals 87 days or more after operation, the frequency of stages 3, 4 and 5 varied considerably, indicating that these changes are by no means constant. A detailed analysis of the changes as seen in roentgenograms is given in table 1.

Deviations from these five distinct stages are represented in figure 1*G* and *H*. *G* shows that folding is not always restricted to the apical end but extends sometimes to the central portion of the inciscor. *H* indicates that the incisors of some rats killed after the same interval (497 days) as those depicted in figure 1*E*, *F* and *G* showed only the changes described for stages 1 and 2. Figure 1*I*, showing the upper incisor of a normal control rat 449 days of age at autopsy, is included for purposes of comparison.

Roentgenograms of whole skulls (shown in fig. 2) allow comparison of the changes occurring after long postoperative periods. Figure 2*A* is a reproduction of the roentgenogram of the skull of a 458 day old normal control rat. The skull of a hypophysectomized rat killed 376 days after the operation (fig. 2*B*) is seen to be distinctly smaller, with the incisors shorter and smaller, than those of the normal control. However, it is interesting to observe that this animal, which presented changes typical of approximately one third of the 27 rats of its group (table 1), did not show any folding of the apical end of upper or lower incisors. The thickening of the labial and the lingual dentin nevertheless was distinct. In another animal, killed 349 days after the operation (fig. 2*C*), a severe apical folding had occurred in the upper incisor but not in the lower. Finally, figure 2*D* shows the skull of an animal 574 days after operation in which the apical foldings and the oblitera-

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EXPLANATION OF FIGURE 1

Roentgenograms of incisors of hypophysectomized female rats taken at different postoperative periods (operation at the twenty-sixth day of age) (3½ times natural size): *A*, postoperative period 12 days; age at autopsy 38 days. Note absence of structural changes. *B*, postoperative period 14 days; age at autopsy 40 days. Stage 1. *C*, postoperative period 87 days; age at autopsy 113 days. Stage 2. *D*, postoperative period 100 days; age at autopsy 126 days. Stage 3. *E*, postoperative period 497 days; age at autopsy 523 days. Stage 4. *F*, postoperative period 497 days; age at autopsy 523 days. Stage 5. *G*, postoperative period 497 days; age at autopsy 523 days. Note that the folding extends over the apical half of the incisor. *H*, postoperative period 497 days; age at autopsy 523 days. Note absence of folding. In spite of the long postoperative period, only stages 1 to 3 can be seen. *I*, incisor of a normal control rat whose age at autopsy was 449 days. Compare it in size with the incisors of the hypophysectomized rats.

TABLE 1.—*Roentgenographic Analysis of Changes of the Incisors of Hypophysectomized Female Rats Following Different Postoperative Periods (Operation at Twenty-Sixth to Twenty-Eighth Day of Age)*

Rats	Post-operative Period, Days	Age at Autopsy, Days	Stage 1: Thickening of Dentin, Labial		Stage 2: Thickening of Dentin, Lingual		Stage 3: Distortion of Apical Curvature Without Folding		Stage 4: Distortion of Apical Curvature With Folding of Upper Incisor		Stage 5: Distortion of Apical Curvature With Folding of Upper and Lower Incisor		Groups Observed by Schour and Van Dyke
			Rats	%	Rats	%	Rats	%	Rats	%	Rats	%	
4	6	32-34	..	...	..	...	..	..	..	..	..	..	
4	8	34-36	..	...	..	...	..	..	..	..	..	..	
4	10	36-38	..	...	..	...	..	..	..	..	..	..	
4	12	38-40	..	...	..	...	..	..	..	..	..	..	
3	14	42	3	100	..	...	..	..	..	..	..	..	
3	16	44	3	100	..	...	..	..	..	..	..	..	
5*	18	72-75	5	100	..	...	..	..	..	..	..	..	
5	30	56-58	5	100	..	...	..	..	..	..	..	..	
5	37	63-64	5	100	..	...	..	..	..	..	..	..	
5	43	69	5	100	..	...	..	..	..	..	..	..	
5	87	114	5	100	4	80	..	..	..	..	..	..	
1	91	119	1	100	1	100	..	..	..	..	..	..	
7	100	126-127	7	100	7	100	1	14	..	..	..	..	Group I; postoperative period 2 to 6 mo.; 8 rats
4	144	170-172	4	100	4	100	2	50	1	25	..	..	
22	206-210	231-237	22	100	22	100	6	27	6	27	1	5	Group II; postoperative period 6 to 10 mo.; 8 rats
4	289-302	315-330	4	100	4	100	..	..	2	50	1	25	
27	316-376	342-404	27	100	27	100	3	11	14	52	4	14	Group III; postoperative period 10 to 15 mo.; 7 rats
1	442	468	1	100	1	100	..	..	..	..	..	..	
6	497	523-525	6	100	6	100	..	..	4	65	1	25	
8	505-519	531-547	8	100	8	100	2	25	5	62	1	12	
10	534-574	560-602	10	100	10	100	1	10	5	50	3	30	
2	617	645	2	100	2	100	..	..	2	100	2	100	
2	645-664	671-692	2	100	2	100	..	..	1	50	..	..	
141	Occurrence of stage after first seen		125	100	98	99	15	16	40	46	13	16	

* This group was hypophysectomized at 57 days of age.

tions of the pulp tissues of upper and lower incisors were extreme. Even though the dental changes vary considerably in figure 2*B*, *C* and *D* the skulls are seen to be approximately the same size.

Histologic Aspects.—Since roentgenograms of hard structures frequently do not disclose minute pathologic changes, the skull of the 26 animals with the longest postoperative periods were prepared for histologic examination. They were selected without consideration of roentgenographic findings. All had lived from 316 to 664 days after the operation and were divided into two groups (table 2); Group I

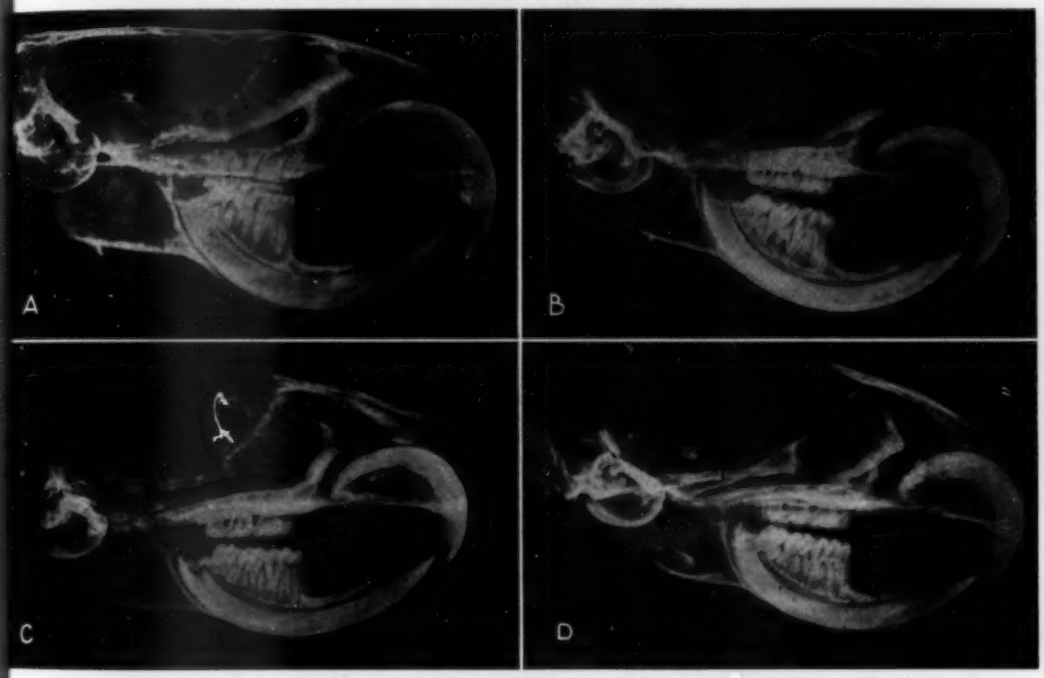

Fig. 2.—Roentgenograms of the right halves of the skulls of normal and hypophysectomized rats (2 times the natural size): *A*, normal control; age at autopsy 458 days. *B*, hypophysectomized rat; postoperative period 376 days; age at autopsy 403 days. Note absence of apical foldings in the upper as well as in the lower incisor. *C*, hypophysectomized rat; postoperative period 349 days; age at autopsy 372 days. Apical foldings occur only in the upper incisor. *D*, hypophysectomized rat; postoperative period 574 days; age at autopsy 603 days. Folding occurs in the lower as well as in the upper incisor.

included 11 animals killed 316 to 442 days after operation and corresponded to the oldest Schour and Van Dyke group with the most advanced changes in the incisors. Group II was composed of 15 even older animals, those which lived 512 to 664 days after the operation.

Central sections of the incisors were examined for thickening of the labial and the lingual dentin, distortion, folding and degeneration of

enamel epithelium, and other general tissue changes. The type and the frequency of pathologic changes are given in table 2.

Photomicrographs of incisors of normal and hypophysectomized rats may be compared in figures 3 and 4. Figure 3*A* shows a central section of an upper incisor of a normal rat which had not received any injection and which was 300 days old at autopsy. The blood supply to the pulp and to the paradental structures was abundant. The odontoblasts were

TABLE 2.—*Histologic Analysis of Changes of the Upper Central Incisors* of Twenty-Six Hypophysectomized Female Rats Following Different Postoperative Periods (Operation at Twenty-Sixth Day of Age)*

Group	Rat No.	Postoperative Period, Days	Age at Autopsy, Days	Stage 1: Thickening of Dentin, Labial	Stage 2: Thickening of Dentin, Lingual	Stage 3: Distortion of Apical Curvature Without Folding	Stage 4: Distortion of Apical Curvature With Folding	Histologic Classification According to Groupings of Schour and Van Dyke	Roentgenographic Analysis According to Table 1: Stages
1†	G2295	316		+	+	—	—	I	2
	G7510	322		+	+	—	—	I	2
	B6347	326		+	+	+	—	I	3
	BH8085	338		+	+	—	+	III	4
	GH4607	342		+	+	—	+	II	4
	W3908	345	342-408	+	+	—	+	II	4
	B3856	347		+	+	—	+	III	4
	BH3790	347		+	+	—	+	II	4
	W6679	351		+	+	+	—	I	3
	W3840	376		+	+	—	—	I	2
	W4120	442		+	+	—	—	I	2
Total 11 rats				11+ = 100%	11+ = 100%	2+ = 18%	5+ = 45%	I 6 = 55% II 3 = 27% III 2 = 18%	
2	W1687	512		+	+	—	+	II	4
	W7715	512		+	+	—	—	I	2
	G7113	510		+	+	—	+	II	4
	G6258	534		+	+	—	—	II	2
	W3841	561		+	+	—	—	II	2
	W3762	561		+	+	+	—	I	3
	B4927	574		+	+	—	+	III	4
	B4923	574	538-600	+	+	—	+	III	4
	W4856	574		+	+	—	—	I	2
	BH01	574		+	+	—	+	III	4
	W56	574		+	+	—	+	II	4
	GH7910	617		+	+	—	+	III	4
	W7650	617		+	+	—	+	III	4
	B3763	645		+	+	—	+	III	4
	G5092	664		+	+	+	—	I	3
Total 15 rats				15+ = 100%	15+ = 100%	3+ = 20%	10+ = 67%	I 4 = 27% II 5 = 33% III 6 = 40%	

* The lower incisors were not studied histologically.
† This group corresponds to group III of Schour and Van Dyke.

active in the apical half, and labial and lingual dentin were deposited uniformly and stained evenly with hematoxylin-eosin. The pulp canal was wide both in the basal zone and in the central portion of the tooth. The ameloblastic layer was normal in its arrangement and distribution from the apex up to the incisal third of the tooth. Owing to the decalcification process, the enamel which originally covered the convex surface of the central incisor was not seen except in the apical tenth, where the new enamel had contained much organic matter. Enamel epithelium

possessed its normal columnar form throughout the apical two thirds; in the incisal third it was reduced to a thin line of flattened cells. The cementum was approximately 3.75 microns thick. (The latter two structures are present only on the concave or lingual side of the rat's incisor.) The bony crypt envelops the apical four fifths of the tooth and affords firm support.

The severe changes occurring in the rat's incisor after a long postoperative period (617 days, stage 4) are demonstrated in figure 3*B*. The pulp canal was almost completely obliterated except in the apical fifth. A severe folding of the labial dentin and enamel and a displacement of Hertwig's sheath had taken place. The lingual dentin also showed slight distortion. The cementum was approximately 11 microns thick (three times as thick as the control); this thickening of cementum was observed histologically in all hypophysectomized rats studied. The paradental bone on the lingual side appeared thin and porous. Enamel formation still continued, though drastically reduced and restricted to the extreme apical region. The few remaining columnar ameloblasts were found only in Hertwig's epithelium sheath. Enamel was absent in the depths of the folds. In these places connective tissue was observed to be in direct contact with dentin. The apical foramen was greatly reduced in size. The tooth was only approximately three fourths the size of the incisor of the control (fig. 3*A*). It will be noted that the dentin stained much darker after hypophysectomy. This was apparently due to an increase in density.

In contrast to these marked changes, figure 4 demonstrates a central incisor of an animal of the same postoperative period (617 days) with only slight deviation from the normal.[6] Folding was completely absent, changes being restricted to partial obliteration of the pulp canal, the result of thickening of the layers of the labial and the lingual dentin, and degeneration of the enamel epithelium down to the apical third. The paradental structures were less vascular and more fibrous than those of the normal rat. This incisor was also only three fourths the size of the normal control (fig. 3*A*).

As the most significant changes were seen at the apex a further analysis of this region is shown in higher magnifications (figs. 5 and 6). Only changes in the upper incisors are illustrated. Since histologic studies were made only of rats living 316 days or more after hypophysectomy, stage 1 could not be demonstrated here.

Figure 5A shows a central section through the apical region of a normal control rat 458 days old. The apical foramen was wide, the

6. The increase in the width of the space at the gingival margins of figures 3*B* and 4 as compared with that of the control in figure 3*A* are artefacts produced in histologic preparation.

Fig. 3.—*A*, upper incisor of a normal rat; age at autopsy 300 days. *B*, upper incisor of a hypophysectomized rat; postoperative period 617 days; age at autopsy 650 days. The pulp canal is almost completely obliterated; there is grotesque displacement of Hertwig's sheath, as well as degeneration of enamel epithelium.

ameloblastic and odontoblastic layers, as well as the enamel and dentin formation, appeared normal. Some enamel matrix could still be seen at the apex. The pulp tissue had an abundant blood supply. Figure 5*B* shows the apical third of the upper incisor of a hypophysectomized rat which was killed 617 days after hypophysectomy.[7] The apical foramen was distinctly narrower; the vascularity was reduced; the labial dentin was definitely thickened; the lingual dentin, only slightly. It may be seen from this illustration that folding of the hard structures may not occur even after postoperative intervals as long as 617 days.

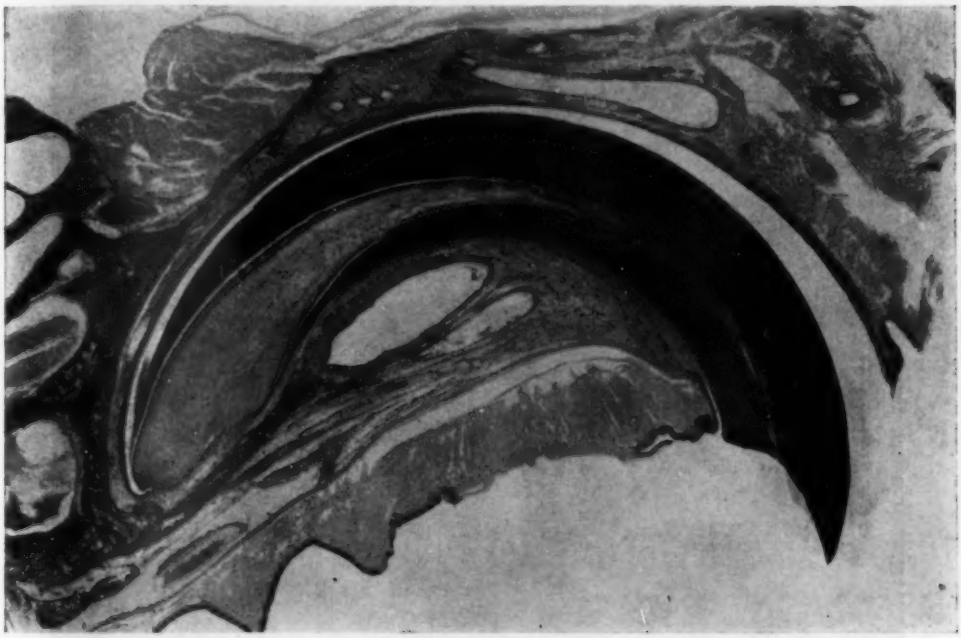

Fig. 4.—Upper incisor of a hypophysectomized rat; postoperative period 617 days; age at autopsy 650 days. Folding is absent in the apical third. Enamel formation is active. The pulp canal is partially obliterated. The tooth is approximately three fourths the size of the normal control *A* in fig. 3.

Figure 5*C* demonstrates the much more marked changes, occurring in this instance after a shorter posoperative period than in the preceding case (331 days). Here there was marked thickening of dentin on both the labial and the lingual sides (stage 2). The extreme apical portion of the labial dentin was slightly wavy; the odontoblasts appeared normal. The ameloblasts had degenerated from the incisal third of the tooth to approximately the apical tenth (*a* in fig. 5*C*). The vascularity of the pulp

7. A higher magnification of the apical region of the incisor is shown in figure 4.

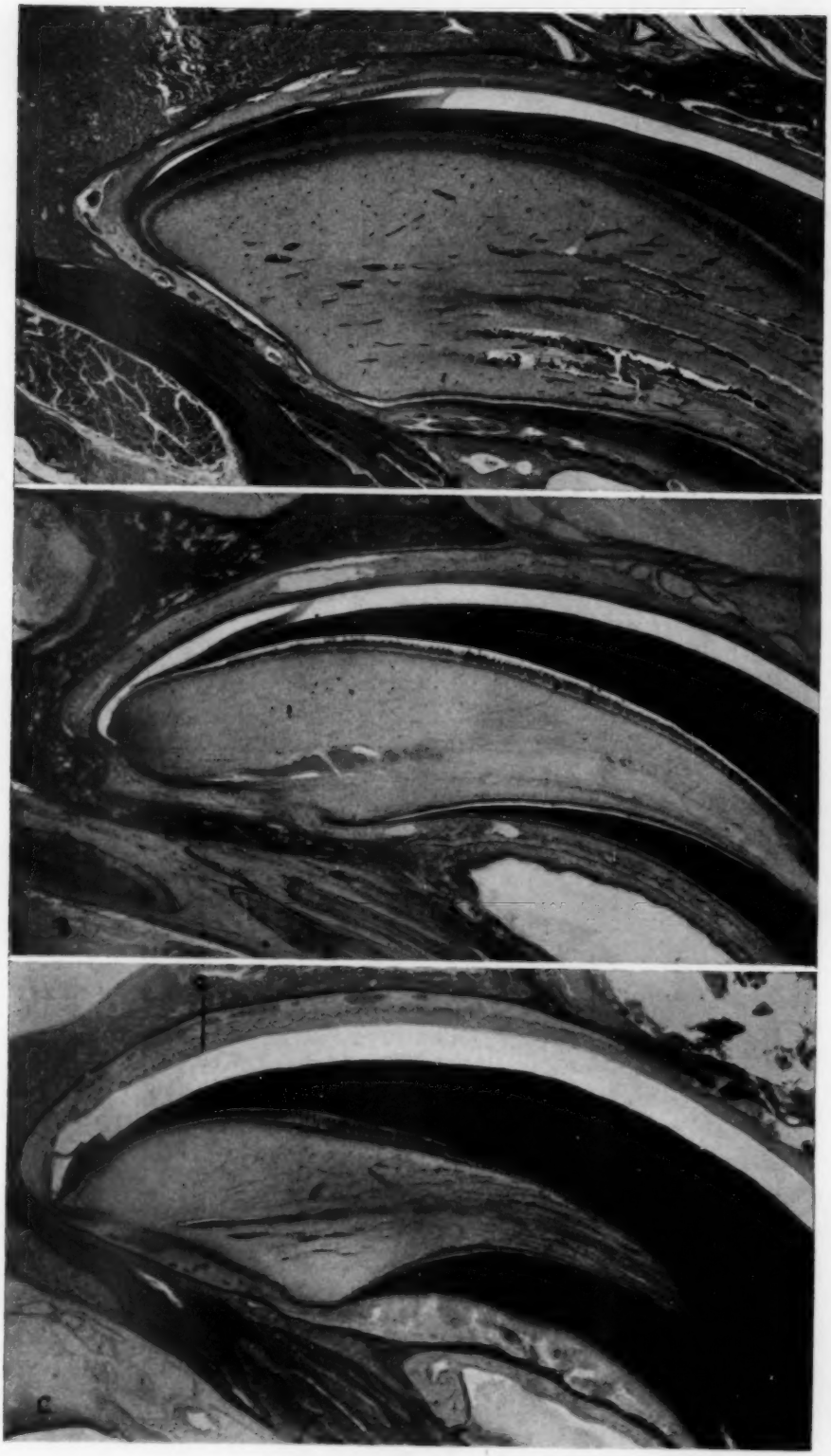

Figure 5
(See legend on opposite page)

and the size of the pulp chamber were reduced to only a fraction of the normal.

The changes characteristic of stage 3 are seen in figure 6*A*. Thickening of the labial dentin was even more pronounced, and a distinct distortion of the labial curvature at the apex was present. The width of the apex was approximately the same as in the preceding animal. The ameloblasts with normal columnar form were restricted to the apical tenth. The pulp chamber and the vascularity of the pulp were greatly reduced when compared with the nomal control of figure 5*A*.

Figure 6*B* shows the distortion of labial dentin and enamel and the multiple areas of enamel hypoplasia (stage 4). Folds were seen throughout the apical third. The ameloblasts had degenerated from the incisal region to the apex. There were only a few small islands of columnar ameloblasts between extensive hypoplastic areas. The vascularity of the pulp was severely restricted. The dentin on the lingual side was thick and ended abruptly at the apex instead of tapering as in the normal. The cementum showed increased thickness.

Figure 6*C* demonstrates the most extreme changes which were observed in the apical region of the upper incisor of the hypophysectomized rat. Labial dentin and enamel were thrown into many overlapping folds. The ameloblasts were flattened or absent. Connective tissue was found in direct contact with the surface of the dentin in the depths of the folds near the apex of the tooth. Hertwig's sheath was markedly displaced, and the apical foramen was reduced to only a small opening. The lingual dentin was not as thickened as in some of the other sections and was irregular in outline. The odontoblasts no longer maintained their orderly distribution as in the normal. They were observed to be shorter and even absent in many areas along the center surface. The cementum was thickened, and the blood supply to the pulp was greatly reduced. The pulp tissue, which is normally very cellular, had become fibrous. The foldings appeared somewhat

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EXPLANATION OF FIGURE 5

High magnifications of apical thirds of upper incisors of normal and hypophysectomized female rats: *A*, normal rat; age at autopsy 458 days. Note the vascularity of the pulp, the extent of the active enamel epithelium and the smooth outlines of the thin labial and lingual dentin. *B*, hypophysectomized rat; postoperative period 617 days. Note the increased thickness of the labial dentin and the restriction of columnar enamel epithelium to the apical one fifth of the incisor. The thickening of the lingual dentin is not extreme. *C*, hypophysectomized rat; postoperative period 331 days. Note the definite thickening of the lingual as well as of the labial dentin, typical for stage 2. Restriction of columnar enamel epithelium to the apical tenth of the tooth is observed at *a*. Note the slight wavy appearance of the apical dentin and enamel on the libial side.

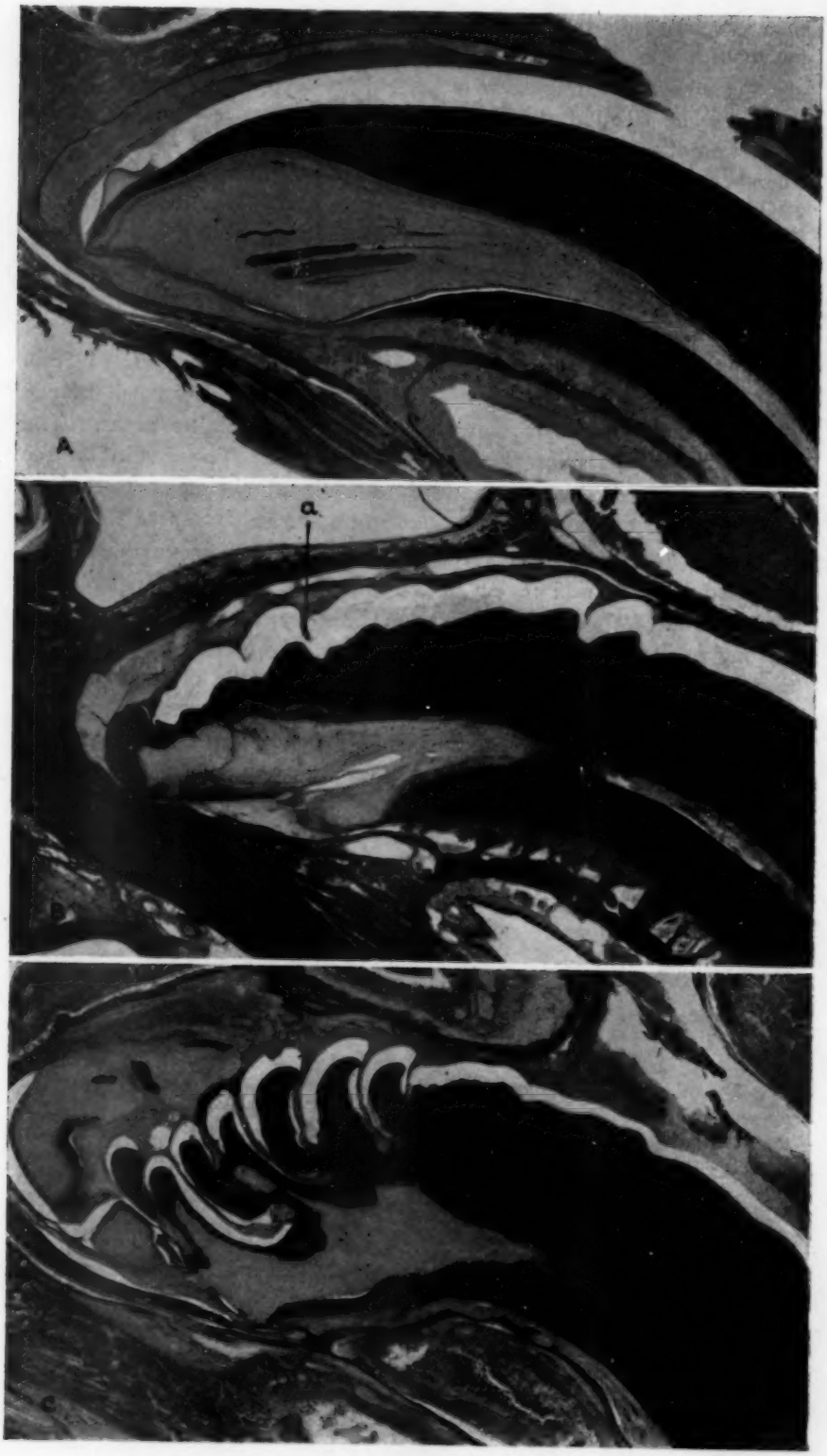

Figure 6

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similar to those described for magnesium deficiency of the rat by Becks and Furuta [8].

A cross section of an upper incisor which yields further information on the nature of the foldings is shown in figure 7. The apical end

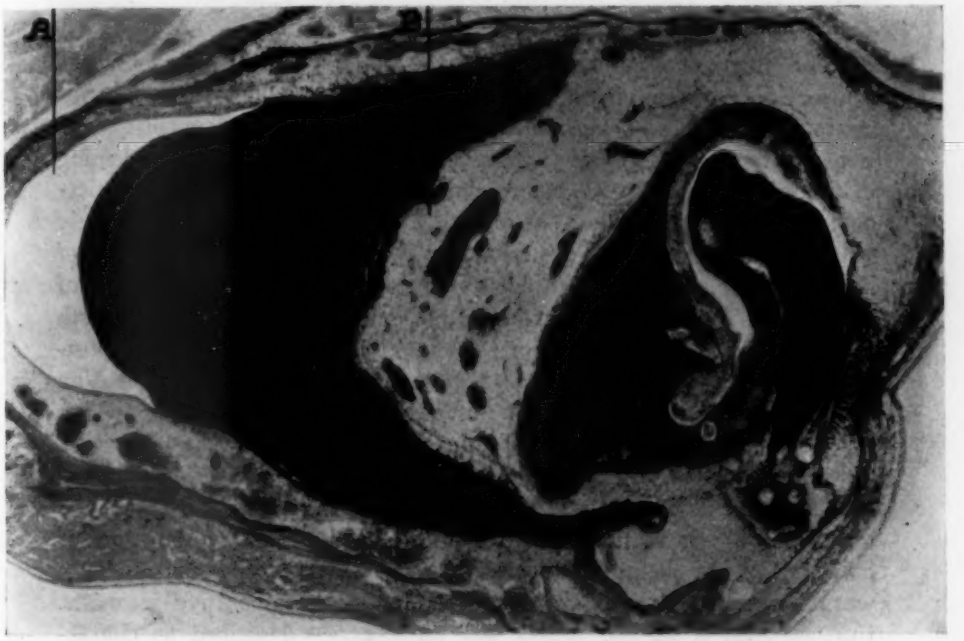

Fig. 7.—Cross section through the apical third of an upper incisor of a hypophysectomized rat with severe folding; postoperative period 574 days. Complete degeneration of the enamel epithelium at this level is seen at *A*, and degeneration of odontoblasts at *B*.

of this upper incisor showed roentgenographically marked folding and other characteristics of stage 4. On the labial side the enamel was

8. Becks, H., and Furuta, W. J.: J. Am. Dent. A. **28**:1083, 1941.

EXPLANATION OF FIGURE 6

High magnifications of apical thirds of upper incisors of hypophysectomized rats: *A*, postoperative period 322 days. Note the thickening of the labial and the lingual dentin with distortion of the curvature of the apical tenth typical for stage 3. *B*, postoperative period 345 days. The enamel and the dentin on the labial aspect have a wavy appearance throughout the apical third, found in stage 4. The enamel epithelium has degenerated to the extreme apical end. The projection of the remains of the enamel epithelium and connective tissue at *a* indicates hypoplasia. The vascularity and the cellular content of the pulp are greatly reduced. *C*, postoperative period 617 days. There is severe folding of labial tissues, typical for stage 4, with extreme displacement of Hertwig's sheath. Note the severe hypoplasia of the enamel, with the surface of the dentin in direct contact with the connective tissue. Some enamel epithelium is still present in the displaced Hertwig's sheath. The vascularity and the cellular content of the pulp are greatly reduced, as in figure 6*B*.

still present (*A* in fig. 7); however, the ameloblastic layer had completely degenerated. The odontoblasts had also disappeared for the main part (*B* in fig. 7). The dentin was seen to fold not only in the labiolingual direction but in the mesiodistal direction.

COMMENT

The roentgenographic study of the teeth from 141 hypophysectomized rats has disclosed no detectable change up to 12 days after operation. The first morphologic deviations from the normal were noted in the incisor 14 days after operation and consisted of thickening of the labial dentin layer (mantel dentin). This was especially noticeable in the posterior half of the tooth and increased progressively with longer postoperative periods. This increase in thickness of dentin led to gradual and in many instances complete obliteration of the pulp. These changes are due not only to disturbance in tooth formation but to retardation in the rate of eruption.[9] Since 63 postoperative days was the shortest period reported by Schour and Van Dyke, the early stages were not described by them.

Thickening of the lingual dentin was seen for the first time 87 days after the operation, but since the next shorter period studied was 43 days, this stage may begin earlier than 87 days. By this time, the labial dentin was markedly thickened, and obliteration of the anterior half of the pulp chamber had been completed. This lingual thickening was characteristic of all animals with longer postoperative intervals. Furthermore, it can be stated that the changes described as characterizing stages 1 and 2 were the only ones which occurred consistently in every hypophysectomized rat after the time of their appearance, 14 and 87 days, respectively. From this it may be said that the thickening of the labial and the lingual dentin and the smaller size of the tooth are the only consistent pathognomonic signs of hypophysectomy which were recognized in the roentgenograms.

The changes which were characteristic of stages 3, 4, and 5, i.e., distortion of the apical curvature without folding, folding of the apical third of the upper incisor without folding of the lower incisor, and finally folding of the posterior portion of both the upper and the lower incisor, are by no means consistent or uniform and therefore are not pathognomonic for hypophysectomy. For instance, after a period of 100 days or more a distortion of the apical curvature without folding occurred only in 15 of 93 animals, i.e., in 16 per cent (table 1). This distortion represents the forerunner of the foldings seen in stages 4 and 5. It was an abrupt change in the outline of the smooth convex

9. Studies of eruption rates in hypophysectomized female rats will be reported separately.

surface of the incisor where the apical end dropped off to form an acute angle with the other portion of the tooth. It could be detected roentgenographically in every instance in which it occurred because of the characteristic deviation from the normal outline of the surface. One gains the impression from histologic observation that this change in curvature at the apical end is an advanced expression of a disproportion in the rate of the eruption and the rate of the active formation of the tooth. The earliest expression of this disproportion was thickening of the labial and the lingual dentin.

The folding of the apical region described as stage 4 (table 1) was also inconstant. It was observed in only 40 of 86 (46 per cent) of the hypophysectomized female rats 144 and 664 days after the operation. In rats which lived from 144 to 210 days after the operation this folding was even less frequent; it was seen in only 7 of 26 animals (27 per cent).

Since Schour and Van Dyke concluded that folding of the apical region of the incisor is a feature pathognomonic of hypophysectomy in rats which survived a period of 6 months and longer and since folding was not found to be a constant characteristic even at much longer postoperative periods, it appeared important to analyze further the incidence of these advanced pathologic changes. The distribution of the animals of Schour and Van Dyke (groups I, II and III) as compared with the animals described in this report is indicated in the last column of table 1.

In comparing the frequency of changes reported by Schour and Van Dyke, based on 23 rats, with that found for the larger group of 71 rats with comparable postoperative intervals used here, the following observations were made:

Among the 17 rats that lived from 87 to 144 days after hypophysectomy (corresponding to group I in the report of Schour and Van Dyke) only 1 animal was found with definite folding of the labial hard structures in the upper incisor (stage 4) yet with no folding of the lower incisor. This represented only approximately 5 per cent of the group of 17 animals. The frequency of folding increased to 30 per cent of the 26 animals which survived 205 to 302 days (corresponding to group II of Schour and Van Dyke), and to approximately 50 per cent of the 28 animals which survived 316 to 442 days (corresponding to their group III).

Schour and Van Dyke also differentiated between various degrees of folding and described folding of the enamel epithelium with definite indentations in the enamel surface as characteristic of their group I (8 rats). In this study such indentations in the enamel were not observed in the group of 17 animals of comparable postoperative age, with the exception of 1 with a definite folding of the apex. Schour and

Van Dyke also demonstrated the folding of the lower central incisor as characteristic of animals in their group II. Among the corresponding 26 rats of this report, only 2 (approximately 8 per cent) showed a folding of the lower incisor. In the age group corresponding to group III of Schour and Van Dyke (316 days or more) the frequency of the labial folding of the lower incisor increased to approximately 15 per cent; these changes did not always occur simultaneously with the folding in the upper incisor.

In this series studies were made of 28 hypophysectomized rats which survived up to 222 days longer than any of those reported by Schour and Van Dyke. Even for this older group it was not possible to state that the advanced changes at the apical end of the upper or the lower incisor were either constant or pathognomonic for complete hypophysectomy of the rat.

Since only rats with the longest postoperative periods were studied histologically, the early stages, 1 and 2, the thickening of labial and lingual dentin, were found in each specimen. As may be seen from table 2, stage 3, the distortion of apical curvature without folding, was observed only twice (18 per cent) in group 1, which corresponds to group III of Schour and Van Dyke as far as postoperative periods are concerned. Stage 4, the most extreme distortion of the apical curvature with folding, was seen only five times (45 per cent). These percentages increased slightly in group II, with postoperative periods of from 512 to 664 days, to 20 per cent and 67 per cent, respectively. In checking the histologic changes against the groupings of Schour and Van Dyke, the interesting picture is revealed (table 2) that their group III, with the most extreme changes, is represented only twice (19 per cent) in our group I, group II three times (27 per cent) and group I six times (55 per cent). Even after longer postoperative periods (table 2, group II) group III of Schour and Van Dyke is found only six times (40 per cent), group II five times (33 per cent) and group I four times (27 per cent). These percentage figures check closely with the roentgenographic analysis (table 1).

It is possible that the greater physical vigor of the strain of rats used in this experiment and the hygienic conditions may account for the late appearance and the lower frequency of the foldings observed in this study.

In comparing the histologic observations of the teeth of 26 animals with the roentgenographic observations (last column in table 2) it was found that with the exception of the earliest displacement of Hertwig's sheath, which is not visible in the roentgenogram, all histologic changes confirmed the roentgenographic classification. This exception was noticed in rat W3762 (table 2, group II).

CONCLUSIONS AND SUMMARY

Roentgenographic and histologic studies of the incisors of 141 female hypophysectomized rats, killed at intervals between 6 and 664 days after the operation, permit the following conclusions:

The earliest change in the upper incisor as seen in the roentgenogram was thickening of the labial and the lingual dentin at the expense of the pulp chamber. This alteration occurred at 14 and 87 days, respectively, after hypophysectomy and was the only constant and pathognomonic symptom observed.

Distortion of the apical labial curvature, distinct folding of the upper incisor alone, and folding of both upper and lower incisors were first seen 100, 144 and 205 days, respectively, after hypophysectomy. None of these symptoms was pathognomonic. The folding of the upper incisors alone was most frequently seen. It occurred in 46 per cent of the hypophysectomized rats 144 or more days after the operation. Folding of the lower incisors was present in only 13 of the 40 rats which showed folding of the upper incisors. It never occurred alone, i.e., without simultaneous folding in the upper jaw. The various degrees of folding were found to be an expression of delays in eruption.

Histologic observations were limited to animals with long postoperative intervals. The most outstanding change in cellular structures occurred in the ameloblastic layer. The regressive changes were not uniform for all animals but were directly proportionate to the severity of the distortion or folding of the apical tissues. Degeneration of the ameloblasts in localized areas led to hypoplasia of the enamel and in some places to agenesia of the enamel so severe as to leave connective tissue in direct contact with the surface of the dentin.

Degeneration of the odontoblasts began in the incisal portion of the incisor and reached the apical end only in animals that showed advanced folding. Marked diminution in the vascularity of the pulp also characterized the advanced changes.

Increase in the thickness of the cementum was observed histologically in the incisors of all hypophysectomized rats and did not vary with the postoperative interval (316 to 644 days after the operation).

RENAL LESIONS IN PORTAL CIRRHOSIS

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PATHOLOGIC changes occurring simultaneously in the liver and the kidneys have been described in a number of disease processes. Heyd,[1] in his classification of "liver deaths," emphasized the occurrence of progressive signs of renal failure culminating in anuria and death. This syndrome has become widely known as the hepatorenal syndrome. Wilensky,[2] Reich[3] and Boyce[4] have reviewed the phenomenon at length. Symptoms of renal failure are frequently seen along with evidence of hepatic disease following use of toxic drugs and chemicals, burns, intestinal obstruction, toxemia of pregnancy, infectious disease, such as Weil's disease and yellow fever, and other conditions. Orr and Helwig[5] emphasized the importance of renal failure following traumatic injury of the liver.

The majority of workers have thought the renal failure to be due to toxic substances acting on the tubular epithelium. These substances are assumed to arise as products of chronic infection, metabolism, or breakdown of liver cells or other tissue and to reach the kidneys in sufficient amounts to cause damage because they are not detoxified by the poorly functioning liver. Lichtman and Sohval,[6] on the other hand, attributed the anuria and the azotemia occurring in hepatic disease to prerenal deviation of fluids.

In cases of the hepatorenal syndrome of the type described by Heyd the pathologic changes of the kidneys have not always corresponded in severity to the clinical manifestations. Although it has been stated that tubular degeneration occurs, there have been few extensive and detailed descriptions of the renal changes. Renal lesions resulting from the action of certain chemical agents which also produce hepatic injury have been

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fairly extensively studied. Lichtman and Sohval [6] described granular, hydropic, vacuolar and fatty degeneration in the convoluted tubules of the kidney in 12 cases of acute and subacute yellow atrophy of the liver. Presumably most or all of the patients were jaundiced, and the renal lesions might be attributed, at least in part, to this factor.

Anatomic changes in the kidney, consisting chiefly of tubular degeneration, resulting in disturbances of renal function and abnormal urinary findings, have been frequently described in marked jaundice.[7] This process has been called bile or cholemic nephrosis. Stewart and Cantarow [8] noted the occurrence of degenerative changes in the renal tubular epithelium in cats and dogs following the administration of relatively small amounts of sodium dehydrocholate. Regeneration of the tubular epithelium occurred in a few days. The effect of naturally occurring bile acids was not determined. Whether renal damage might be produced by excessive urinary excretion of bile acids in cases of liver disease, even without jaundice, has not been definitely determined but does not appear likely to us.

Symptoms of renal failure and renal lesions occurring in cirrhosis of the liver have not been widely emphasized. Albuminuria, abnormal urinary sediment and azotemia have been reported even in the absence of jaundice,[9] but we know of no detailed studies of the anatomic changes in the kidneys of patients with cirrhosis of the liver.

In recent years, studies of the effects on experimental animals of diets deficient in certain substances have thrown additional light on the relationship of degenerative changes in the liver and the kidneys. Following the observations of Allen and associates [10] and Fisher [11] in 1924 that in depancreatized dogs treated with insulin fatty livers developed, Best and associates [12] found that the fatty changes could be prevented by choline. It was observed that casein (containing methionine),[13] methionine [14] and certain other substances were lipotropic, and this was explained by du Vigneaud [15] as due to the ability

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of these substances to furnish labile methyl groups for the synthesis of choline. György [16] recently reviewed the subject of the production of hepatic injury and cirrhosis by dietary means.

In 1939 Griffith and Wade [17] noted the occurrence of renal tubular degeneration with cortical congestion and hemorrhages within six to eight days in young rats fed diets deficient in choline and other lipotropic factors. Impairment of renal function and albuminuria were also noted in these animals. Renal lesions produced in rats by choline deficiency were further studied by Christensen.[18]

Since, more and more, a role of deficiency of lipotropic substances in degenerative disease of the liver in man is being suspected,[19] it seemed to us that renal lesions due to the same deficiencies and similar to those produced in the rat by choline deficiency might be found in these cases. One might expect that the renal lesions would be more prominent in the cases of acute disease, that is in acute and subacute necrosis of the liver. However, since nearly all of these patients are jaundiced, and since tubular degeneration has been attributed to jaundice, we decided to study the renal changes in cases of portal cirrhosis, many of which are not complicated by jaundice.

METHOD OF STUDY

Twenty-five cases of portal cirrhosis were selected from the autopsy material of the last ten years at Parkland Hospital. Certain cases were excluded from the series because of complicating renal disease of an inflammatory or a vascular nature. Others were discarded because of severe infection, since it is known that tubular degeneration occurs under this circumstance. Instances of obstruction of the biliary system were also excluded.

Portal cirrhosis was recognized on the basis of nodular regeneration of the hepatic parenchyma accompanied by proliferation of periportal fibrous tissue. Twenty-two of the cases were considered to be of the Laennec type, while 3 were classified as instances of toxic cirrhosis because of evidence of recent or old extensive hepatic necrosis. In most cases the cirrhosis was recognized grossly without difficulty, but in a few cases nodular regeneration was slight and identified with certainty only by microscopic study.

In each case sections of liver and kidneys stained with hematoxylin-eosin were studied microscopically. In 9 cases sudan IV stain for fat, Mallory's aniline blue connective tissue stain and congo red stain were utilized. Clinical records and autopsy protocols were studied for accessory data.

CLINICAL AND GROSS PATHOLOGIC DATA

Many of the patients were admitted in coma or following massive hemorrhge, and it was often impossible to obtain adequate historical data. Most of the patients had had signs or symptoms pointing toward the diagnosis of cirrhosis for from

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two days to five years. Nine patients had jaundice which was of moderate intensity in most of them, but icterus indexes were distributed from 10 to 200. Sixteen had ascites. Eight patients had massive bleeding from esophageal varices, and 7 died from this cause. Fifteen apparently died in coma, presumably as a result of hepatic insufficiency, whereas the remaining 3 died from miscellaneous causes unrelated to the cirrhosis.

Serologic tests for syphilis were positive in 5 of 23 cases. Blood urea was determined in 9 cases, and in only 1 was it normal; in 1 case of cirrhosis with superimposed acute necrosis it was decreased, and in the remaining 7 cases it was slightly to moderately elevated. The level of total serum proteins was determined in 10 cases and was revealed to be subnormal in the majority, and in 5 of these cases the albumin-globulin ratio was definitely inverted. Urinalyses, performed in 20 cases, showed albuminuria present in 60 per cent.

No detailed dietary histories were available. Five of the patients were known to have had chronic alcoholism. Three had received arsenical therapy for syphilis, and 3 received mercurial diuretics without evident toxic reactions.

In the majority of cases the gross characteristics of the liver were typical of portal cirrhosis. Five livers weighed less than 1,000 Gm. Ten weighed 1,000 to 1,500 Gm., 5 between 1,500 and 2,000 Gm., and 3 weighed more than 2,000 Gm. Three showed marked fatty changes, and in 3 cirrhosis was complicated by primary carcinoma.

The spleen in most cases was slightly to moderately enlarged. Two of the spleens were small, weighing less than 100 Gm. Nine weighed between 130 and 180 Gm. and 12 between 215 and 650 Gm.

In nearly all cases the kidneys appeared essentially normal. In 2 cases the individual kidneys weighed a little more than 200 Gm. In a few instances the cortex was slightly swollen and pale. Some of the kidneys revealed fine granularity after stripping of the capsule, presumably due to patchy areas of dilated or hypertrophied tubules, and an occasional kidney had small depressed cortical scars. The ureter and the pelvis of 1 kidney were dilated without obvious cause, and the pelvis of 1 kidney contained a small stone, without obstruction.

Five patients were found at autopsy to have mild hypostatic bronchopneumonia. Three patients had primary carcinoma of the liver, 2 had bronchogenic carcinoma and 4 patients had carcinoma of the following regions, respectively, gallbladder, esophagus, breast and face (epidermoid, squamous cell type). In none of these was the additional pathologic process considered to play a significant part in the illness or the death of the patient.

HISTOLOGIC OBSERVATIONS

Glomerular Lesions.—Minor alterations in the glomeruli were common in this series. In 19 cases there were scattered, completely hyalinized glomeruli, occurring in areas without other significant sclerotic changes. In 10 cases there were changes described as periglomerular fibrosis. This consisted of actual fibrous tissue proliferation and elaboration of collagenous intercellular material outside the basement membrane. Often this process was localized to one side of the glomerulus, but usually a complete ring of fibrous tissue extended around the glomerulus, which was itself often atrophic. In most of these altered glomeruli there was also hyaline thickening of the parietal and occasionally of the visceral basement membrane. In every case in which there was periglomerular fibrosis there was also some degree of arterial or arteriolar sclerosis. Because of this association it seems likely that periglomerular fibrosis and hyaline thickening of the glomerular

basement membrane were due to arterial and arteriolar sclerosis, thus resembling the ischemic changes in the glomeruli described by Kimmelstiel.[20] Furthermore, there was no apparent correlation between the occurrence or severity of the glomerular changes and the tubular degeneration, atrophy and regeneration noted in the following section. It is of some interest, however, that Christensen[18] described periglomerular fibrosis and thickening of the basement membrane of the glomeruli in rats which had been fed diets deficient in choline.

In many cases in which there was marked tubular degeneration the glomerular spaces contained pink protein precipitate.

Tubular Changes.—1. Types and Incidence of Lesions Encountered: The most common lesion was granular swelling (fig. 1 *A*) of the convoluted tubules. This was noted in some degree in every case but was quite prominent in 18 of the 25. In the other 7 cases either slight diffuse or focal swelling of epithelial cells was revealed. Other tubular changes, consisting of atrophy, dilatation and regeneration, occasionally masked the appearance of the granular swelling. The proximal tubules were most strikingly involved. Henle's loops and the distal convoluted tubules showed similar but much less marked alteration. The process was focal or diffuse and consisted of marked enlargement of the cell bodies, with prominence of cell outlines and cloudiness or fine to coarse granularity of the cytoplasm. The granularity was often uniform throughout the cell body, but in some instances the granules at the tips of the cells were sharper and larger. The tips of the cells and usually the brush borders were well preserved. The lesion described here was indistinguishable from that which is well known as acute parenchymatous degeneration or cloudy swelling.

In 10 cases there was also hydropic swelling of the cells of the proximal convoluted tubules. This occurred in those cases in which granular swelling was marked. It consisted of a greater degree of enlargement of the cells with the appearance of clear areas where the granules appeared to be separated from one another by watery cytoplasm.

In 7 cases small vacuoles were noted in the proximal convoluted tubules. Hydropic swelling was present in all these 7 cases except 1. The vacuoles were most numerous at the bases of the cells but occurred to some extent over the entire bodies of the cells, giving them a foamy appearance. This vacuolation was often marked in the straight segments of the convoluted tubules. Fat stains demonstrated the vacuoles to be fat droplets in most instances, but in some areas the vacuoles were fat-free. With this stain, fat droplets were found to be most numerous at the bases of the cells, where they were often arranged in fine parallel rows extending longitudinally in the cells. Fat droplets were less frequently noted in the cells lining Henle's loops, and here they tended to be larger and less numerous than in the convoluted tubules.

Hyaline droplets (fig. 1 *B*) were found in the proximal convoluted tubules in 5 cases. They occurred in focal groups of tubules of the same nephron unit. The droplets were located mainly in the tips of the cells, were brightly eosinophilic and hyaline in structure and did not stain with congo red. There appeared to be no correlation between the occurrence of hyaline droplets and the severity of other tubular changes.

In 10 cases there was dilatation of the convoluted tubules, with the lining being converted to a thin, atrophic-appearing epithelium (fig. 1 *C*). In the most characteristic form these dilated tubules were found scattered throughout the cortex in focal areas involving several tubular loops of the same nephron unit. The out-

20. Kimmelstiel, P.: Am. J. Path. **11**:483, 1935.

side diameter of these tubules was increased, but the inside diameter was even more increased because of the thinning of the lining cells. These dilated tubules occasionally enclosed hyaline casts or protein-containing fluid. In a few cases dilatation of the convoluted tubules was diffuse and was not so marked as in the focal lesions. It appeared to be almost entirely due to atrophy of the lining cells with widening of the lumen.

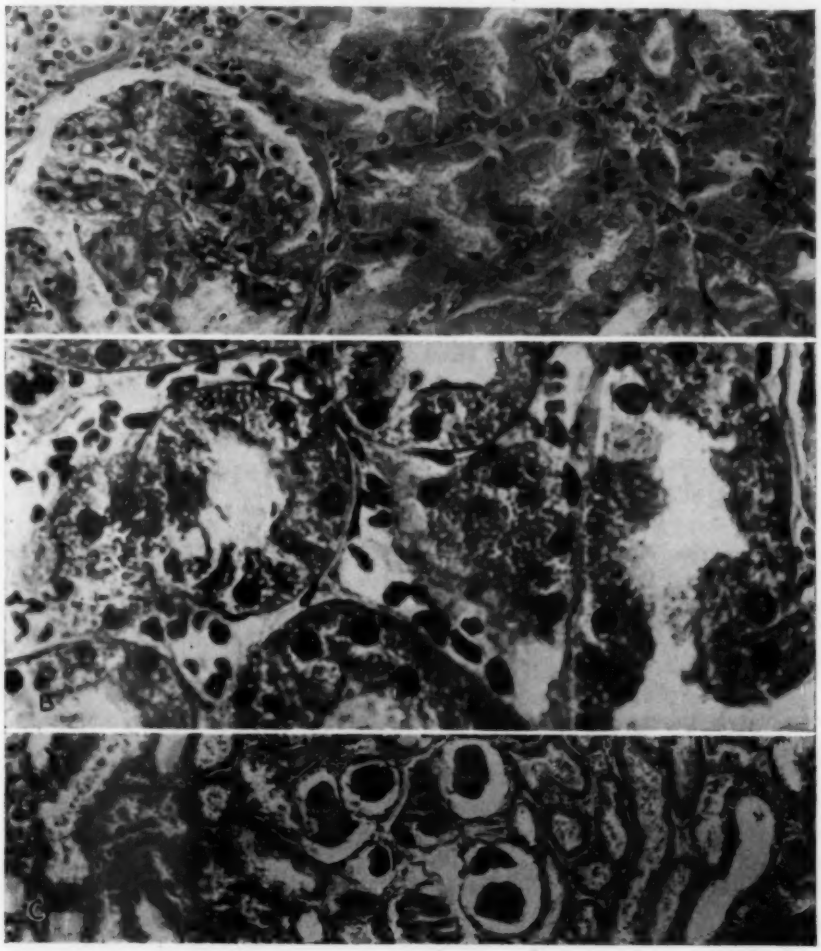

Fig. 1.—*A*, section of kidney showing granular swelling of the epithelium of convoluted tubules and periglomerular fibrosis.

B, hyaline droplet formation in swollen epithelial cells of convoluted tubules.

C, section of kidney revealing focal granular swelling, dilatation and epithelial atrophy and, in addition, regeneration of epithelial cells of convoluted tubules. Hyaline casts are seen in dilated tubules.

In close association with dilatation and atrophy, 7 cases revealed certain changes in the convoluted tubules which seemed to indicate regeneration of the epithelial cells (fig. 2 *A* and *B*). Some dilated tubules appeared to be denuded of epithelium.

Others were lined by low columnar or cuboidal epithelium in which the nuclei were hypertrophied and hyperchromatic. The nuclei were also more numerous in these tubules, and mitoses were observed occasionally. In some instances there were scattered multinucleated syncytial masses in the epithelial lining. The cytoplasm of these regenerating cells was scanty and slightly basophilic. In addition to the dilated tubules with regeneration of epithelium there were frequent groups of tubules in the cortex in which the lumens were small and collapsed, which appeared to be lined by regenerating elements similar to those just described.

No consistent changes were noticed in the interstitial tissue of these kidneys. The collecting tubules were occasionally somewhat dilated, particularly in focal areas, but were otherwise unaltered.

2. Pattern and Possible Evolution of Renal Lesion: With respect to the renal changes, these cases would seem to fall into two general histologic groups. In one group, including the majority of cases, the picture was that of acute tubular injury, consisting of granular swelling, hydropic degeneration, fatty and vacuolar degeneration and presence of hyaline droplets in the convoluted tubules. These changes occurred focally or diffusely and in variable degrees of intensity. In this group the changes were thus characteristic of the acute tubular degenerations sometimes referred to as the acute nephroses.

In the other group the renal changes were of a more chronic nature. The degenerative phenomena were present but less marked, and the predominating lesion consisted, instead, of focal or diffuse dilatation of the convoluted tubules. This was accompanied by atrophy and evidence of regeneration of the tubular epithelium. These tubular changes are indicative of a longer-standing tubular injury with added reparative processes.

Analysis of the various lesions of the kidney revealed what appeared to be a transition from the acute type of tubular swelling to the chronic tubular lesions just described. Many kidneys showed the two processes occurring simultaneously in different areas. Where tubular dilatation with atrophic epithelium was extensive, the acute degenerative lesions were not marked. Regenerative epithelium seemed to replace previously degenerated epithelial cells. It is believed that the first stage of renal tubular injury is that of acute swelling with or without hydropic, vacuolar and fatty degeneration. Following this stage three courses might conceivably be followed: First, there might be complete regression of the degenerative phenomena with return to a normal morphologic state; second, cytoplasm might be partially lost into the lumen from the swollen cells, followed by a state of progressive restitution by cytoplasmic regeneration; or, finally, the cells might undergo necrosis, be removed by disintegrating into the lumen or by autolysis, and in turn, be replaced by regenerating epithelial cells. The morphologic characteristics of the second and the third course are not distinguished with certainty at present.

CORRELATION OF ACTIVITY OF CIRRHOSIS AND SEVERITY OF RENAL LESIONS

An attempt was made to grade the activity of the cirrhotic process in each case, grades of from 1 to 4 plus being used and degenerative changes in the parenchymal cells, fatty infiltration, regeneration, fibrosis and lymphocytic infiltration being taken into consideration as criteria (fig. 2 *C* and *D*).

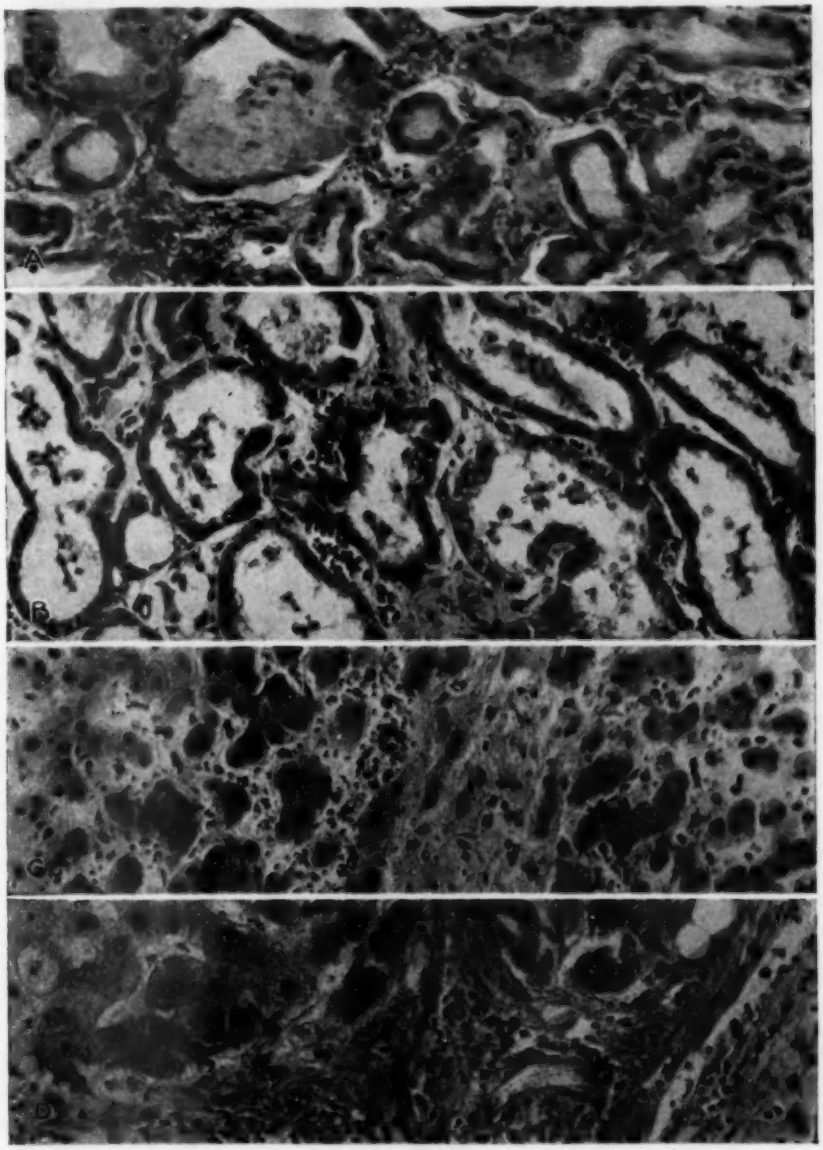

Fig. 2.—*A*, focal tubular dilatation. Note the markedly dilated tubule lined partly by granular swollen cells and partly by flattened cells. Regeneration of epithelium is prominent in the small tubules.

B, marked diffuse dilatation with flattening of lining cells and some evidence of regeneration of the epithelium of the convoluted tubules.

C, cirrhosis of the liver showing slight degree of activity as far as regenerative and inflammatory changes are concerned.

D, cirrhosis of the liver with markedly active regeneration, considerable lymphocytic infiltration and focal fatty metamorphosis. A moderately high degree of activity of cirrhosis is seen.

The severity of the renal changes in each case was graded in a similar manner without reference to the grade of activity of the cirrhosis. In grading the severity of the renal changes the degree of granular swelling of the epithelial cells of the convoluted tubules was regarded as the most important criterion. However, tubular atrophy, dilatation and regeneration, vacuolation of tubular cells, hydropic degeneration and hyaline droplet formation were also noted, and when these changes were present in a significant degree the grade of the granular swelling was increased to obtain the final grade of the renal lesions.

In order to determine whether there is any correlation between the activity of the cirrhotic process and the severity of the renal lesions, a

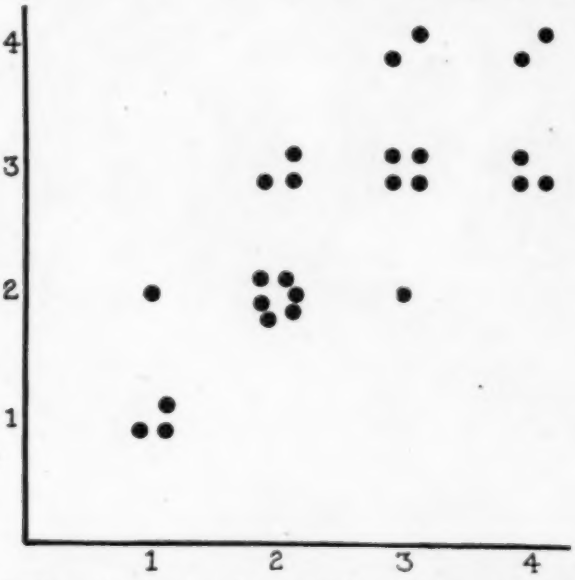

Fig. 3.—Scattergram showing the correlation between activity of cirrhosis (grades 1 to 4 indicated at bottom of chart) and severity of tubular changes (grades 1 to 4 indicated at left).

scattergram was made (fig. 3), the activity of the cirrhosis being used as abscissa and the severity of the renal lesions as ordinate. It can be seen from the graph that there is a definite correlation between the two functions; that is, the more active the cirrhosis, the more severe are the renal lesions. This would seem to provide further evidence that the renal lesions encountered in these cases are related in some way to the cirrhosis itself.

RELATIONSHIP OF JAUNDICE TO THE RENAL LESIONS

Since renal tubular degenerative changes are known to occur in jaundice, supposedly due to the effect of bile salts on the epithelial cells,

it should be considered whether the renal lesions in these cases are to any extent dependent on the presence of jaundice. Table 1 shows the number of cases with each grade of tubular degeneration among the cases of cirrhosis with and the cases without jaundice. It will be noted that even the severer grades of tubular degeneration are seen in the cases without jaundice as well as in those with jaundice. However, the average grade of tubular degeneration is greater in the cases with jaundice. This does not necessarily mean that jaundice per se is even partially responsible for the renal changes, however. Since the activity of the cirrhosis is in general greater in the cases with jaundice, it may be that the extent and the severity of the hepatic disease determine the magnitude of the renal changes. The extent of the renal changes seems to be in proportion to the activity of the cirrhosis irrespective of the presence or the absence of jaundice.

COMMENT

Causes of the Tubular Lesions.—The various degenerative lesions which have been observed in the convoluted tubules and loops of Henle

TABLE 1.—*Relationship of Jaundice to Tubular Degeneration*

		Cases with Given Grade of Tubular Degeneration			
	Cases	1	2	3	4
Cirrhosis with jaundice	9	0	1	6	2
Cirrhosis without jaundice	16	3	7	5	1

appear to be related in some way to the cirrhosis. Disease processes unrelated to cirrhosis can be excluded as a causative factor in most cases of the series. The possibility that jaundice plays an important part appears unlikely. Doubt has already been expressed that tubular injury might be produced even in cases without jaundice by excessive elimination of bile acids in the urine in cirrhosis. Renal anoxia has been suggested as a cause of the tubular degeneration and impairment of renal function occurring in a variety of conditions,[21] and it cannot be entirely eliminated as a possible factor in the production of the lesions observed in some of the cases of the present study.

In view of the fact that Griffith and Wade [17] and Christensen [18] have described similar tubular degeneration occurring simultaneously with hepatic injury in the rat under conditions of experimental choline deficiency, it seems to us that the tubular degeneration observed in this series of cases might be explained on the basis of a similar mechanism; that is, the renal injury is produced by the same factors which produce

21. Macgraith, B. G.; Havard, R. E., and Parsons, D. S.: Lancet **2**:293, 1945.

the hepatic injury. It is difficult or impossible to prove even in animals that the renal damage is a result of the factors which cause the hepatic injury rather than a result of the hepatic injury itself. Dietary deficiency of lipotropic substances and circumstances which increased the requirement of or interference with the action of lipotropic substances seem to be important factors but are not the only ones which play a part in the production of cirrhosis and injury of the liver.[22]

Significance of Renal Lesions.—Most observers have believed acute parenchymatous degeneration as it occurs in the convoluted tubules to be a mild type of cellular injury which is reversible.[23] Others [24] have maintained that it constitutes a morphologic expression of increased function or of stimulation. There seems to be little question, however, concerning the significance of hydropic, fatty and vacuolar degeneration of the tubular cells. These changes are accepted to indicate a more or less severe form of cellular injury.

Hyaline droplets have been noted in the renal epithelium in a wide variety of conditions. Terbrüggen [25] found an incidence of 66 per cent

TABLE 2.—*Relationship of Jaundice to Albuminuria*

		Cases with Given Degree of Albuminuria		
	Cases	0	Trace to +	++ to ++++
Cirrhosis with jaundice	9	3	2	4
Cirrhosis without jaundice	11	5	3	3

in autopsies. He was convinced that these droplets represented a type of alteration different from acute parenchymatous degeneration, a conviction with which our observations are in agreement. Smetana and Johnson [26] concluded from their studies on the salamander that hyaline droplets represent protein stored in the tubular epithelium and that the protein is derived from the tubular urine. In our cases we have not been able to determine what the exact significance of hyaline droplets is, but we believe that it is not directly related to other degenerative lesions.

The relationship of the degenerative tubular lesions to the albuminuria is a matter of considerable interest. The incidence and the severity of albuminuria could not be definitely correlated with the presence or the absence of jaundice, although there is some suggestion in table 2 that

22. Moon, V.: Arch. Path. **18**:381, 1934.

23. Karsner, H. T.: Human Pathology, Philadelphia, J. B. Lippincott Company, 1942.

24. Davidman, A., and Dolley, D. H.: J. M. Research **42**:515, 1921.

25. Terbrüggen, A.: Beitr. z. path. Anat. u. z. allg. Path. **86**:236, 1931.

26. Smetana, H., and Johnson, F. R.: Am. J. Path. **18**:1029, 1942.

the albuminuria is intensified in the presence of jaundice. As Ratnoff and Patek [27] indicated, there is some difference of opinion concerning the incidence of albuminuria in cases of portal cirrhosis, but that it does occur, unexplained by other causes, is certain. Ratnoff and Patek stated that "apparently albuminuria occurs but there is no evidence that this is the result of anything more than congestion of the kidney resulting from the pressure of ascitic fluid on the renal vein." In our cases we find no evidence of marked hyperemia of the kidneys, gross or microscopic. We are inclined, therefore, to interpret the albuminuria as being due to the accompanying parenchymal injury of the kidney rather than to passive hyperemia. The albuminuria might be explained by a possible increase of permeability of the glomerular membrane or by an impairment of tubular reabsorption of the protein of the glomerular filtrate according to the concept of Dock.[28]

We are not able to say whether the oliguria and the azotemia seen frequently in this series of cases were due to the renal lesions or were prerenal in origin. Neither do we know what the end stage of the renal lesion is. There is no evidence that it progresses to a stage comparable to chronic glomerular nephritis or leads to clinical hypertension, but this is an interesting subject for speculation. It also seems possible that the renal lesions might predominate in some cases, giving a picture of renal disturbance with little or no evidence of hepatic disease.

SUMMARY

Pathologic changes occurring simultaneously in the liver and the kidneys under clinical conditions, and similar lesions produced experimentally in animals by means of diets deficient in lipotropic factors, have been described in the literature.

Twenty-five cases of relatively uncomplicated portal cirrhosis were studied by us to determine whether renal lesions were consistently present.

Degenerative changes of the convoluted tubules and to a lesser extent of the loops of Henle, consisting of granular swelling with or without hydropic, vacuolar and fatty degeneration and hyaline droplet formation, were encountered to some degree in all cases. In some cases evidence of a more chronic form of renal injury was revealed, characterized by dilatation, atrophy and regeneration of the convoluted tubules. Glomerular changes consisting of periglomerular fibrosis and hyaline thickening of the basement membrane were observed, but these could not be definitely attributed to the cirrhosis. Albuminous precipitate was frequently observed in the glomerular spaces.

27. Ratnoff, O. O., and Patek, A. J.: Medicine **21**:207, 1942.
28. Dock, W.: New England J. Med. **227**:633, 1942.

Albuminuria was present in 60 per cent of the cases, and this was attributed to renal parenchymal injury resulting in either an increase of glomerular permeability or a decrease of tubular function.

A direct correlation was demonstrated between the activity of the cirrhotic process and the severity of the renal lesions.

All degrees of tubular degeneration were observed in the cases with no jaundice as well as in those with jaundice, and the correlation between the activity of the cirrhosis and the degree of tubular injury was equally good in the two groups, although in general the severity of the tubular degeneration was somewhat greater in the group with jaundice.

Although it cannot at present be said with certainty, we believe that the renal changes described in these cases and in certain other types of cases in which hepatic and renal lesions occur simultaneously might result not directly or indirectly from injury of the liver but rather from the same factors which produce the injury of the liver.

TRANSITORY PULMONARY INFILTRATIONS (LOEFFLER'S SYNDROME) IN RABBITS

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IN 1932 and again in 1936 Löffler reported on a transitory pulmonary disease characterized by a dearth [1] of clinical manifestations, by eosinophilia and by characteristic pneumonic infiltrations roentgenographically. Clinically, some of his patients presented general malaise and an irritating cough productive of a slight amount of yellowish mucoid sputum which was poor in cells and occasionally contained a few eosinophils. Physical examination was often disappointing but sometimes disclosed scattered rales and a fine friction rub. The leukocytes were increased to as much as 15,000 per cubic millimeter of blood and the sedimentation rate to 15 mm. per hour. In about one third of the cases there were no signs or symptoms, the disease being discovered by routine roentgenograms. These revealed unilateral or bilateral, single or multiple, homogeneous or spotty, sharp or ill defined shadows which appeared and disappeared in from three to eight days. Recovery ensued in all his cases, and for this reason the nature of the shadows was not determined. Löffler did not ascertain the cause of the disease in his 51 cases but believed that the lesion was a specific tissue reaction probably produced by a variety of antigens and comparable to erythema nodosa of the skin.

Although numerous case reports and several reviews have appeared in the literature since Löffler's publications, few [2] have added appreciably to his classic description. While most authors agree that the disease is an allergic response on the part of the lungs, the views regarding the causative allergens vary. Probably the most frequently mentioned are animal parasites, including such organisms as Ascaris lumbricoides,[3]

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From the Clinical Laboratories and Department of Radiology, Jefferson Medical College Hospital.

1. Löffler, W.: Beitr. z. Klin. d. Tuberk. **79**:368, 1932; Schweiz. med. Wchnschr. **66**:1069, 1936.

2. (*a*) Freund, R., and Samuelson, S.: Arch. Int. Med. **66**:1215, 1940. (*b*) Peabody, J. W.: Dis. of Chest **10**:391, 1944.

3. (*a*) Müller, R. W.: Deutsche med. Wchnschr. **64**:1286, 1938. (*b*) Barker, C. S.: Canad. M. A. J. **40**:494, 1939. (*c*) Beck, L. C.: Hawaii M. J.

(Footnote continued on next page)

Strongyloides stercoralis,[4] Fasciola hepatica and amebas.[5] Mycobacterium tuberculosis has been considered to be the etiologic agent by some,[6] but this has been denied by others.[7] More recently, many authors have stressed the association of the syndrome with bronchial asthma [8] and so have not only definitely established an allergic basis for the disease but indicated one of the common routes of invasion of the allergen, namely, the respiratory tract. In this connection two specific reports appear to be pertinent. The first was that of Engel,[7b] who stated that in China every year in May and June, when the privet flower blooms, there occurs a cough with expectoration of canary yellow sputum known as the "privet cough." The author himself had the cough on several occasions, at which times studies revealed the typical syndrome outlined by Löffler. Although the privet plant is said to produce little pollen in the air, the seasonal occurrence of the symptoms strongly suggests sensitivity to this allergen. A second pertinent report was that of Dickson,[9] who described the syndrome as observed in 2 laboratory workers suffering from coccidioidomycosis. Dust from dishes on which this fungus was growing was seen to permeate the air when the covers were removed, and it was the author's opinion that the infection was contracted by inhalation of this material.

Because patients with this disease do not as a rule die, most of the knowledge regarding its pathologic character has been speculative. Löffler [1] merely stated that it was a specific tissue reaction. Engel [7b] and later Baer [10] considered the condition as edema of the lungs, Soderling [8a] expressed the belief that the shadows were due to stagnation of secretion which combined with bronchiolar spasm to produce collapse of the lung, whereas Kartagener [11] and Maier [7a] regarded the lesion as true eosinophilic pneumonia. In considering more tangible evidence

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1:361, 1942. (*d*) Meyenberg, H.: Schweiz. med. Wchnschr. **72**:809, 1942; abstracted, J. A. M. A. **121**:626, 1943.

4. Berk, J. E.: J. A. M. A. **127**:354, 1945.

5. Hoff, A., and Hicks, H. M.: Am. Rev. Tuberc. **45**:194, 1942.

6. Leitner, J.: Beitr. z. Klin. d. Tuberk. **88**:388, 1936. Karan, A. A., and Singer, E.: Ann. Int. Med. **17**:106, 1942. Peabody. [2b]

7. (*a*) Maier, C.: Helvet. med. acta **10**:95, 1943; abstracted, J. A. M. A. **123**:869, 1943. (*b*) Engel, D.: Beitr. z. Klin. d. Tuberk. **87**:239, 1935.

8. (*a*) Soderling, B.: Arch. Dis. Childhood **14**:22, 1939. (*b*) Harkavy, J.: Arch. Int. Med. **67**:709, 1941; (*c*) J. Allergy **14**:507, 1942. (*d*) Hennell, H., and Sussman, M. L.: Radiology **44**:328, 1945. Maier.[7a]

9. Dickson, E. C.: J. A. M. A. **111**:1362, 1938.

10. Baer, A.: Ohio State M. J. **37**:960, 1941.

11. Kartagener, M.: Schweiz. med. Wchnschr. **72**:862, 1942; abstracted, J. A. M. A. **121**:893, 1943.

of the pathologic process, the report by Smith and Alexander [12] of a case of the syndrome coming to necropsy must be questioned. Although their patient may have had the disease several months before death a subsequent leukocyte count of 45,600 per cubic millimeter of blood with 97 per cent lymphocytes strongly suggests lymphatic leukemia. Meyenberg's [8d] publication is also open to question because he did not furnish proof of the transient nature in any of his 4 cases. In 1 case he described two small pulmonary foci that contained many eosinophils. In 2 cases there were bronchitis, bronchiolitis and bronchopneumonia, and in 1 case, lobar pneumonia. The paper by Harkavy [8c] on 15 cases of bronchial asthma is the first to furnish acceptable data on the pathologic nature of the pulmonic lesions. At least 3 of the 4 cases that came to autopsy might be considered as typical instances of Löffler's syndrome. In them he noted (1) eosinophilic infiltration, edema and hyalinization of the basement membrane of the bronchial mucosa, (2) infiltration of the alveolar septums with eosinophils, polymorphonuclear leukocytes and lymphocytes, (3) thrombosis of the small vessels and (4) congestion, edema, atelectasis and emphysema of the avleoli. In another case of the syndrome associated with bronchial asthma that came to necropsy Broch [13] also described pulmonary congestion, edema and diffuse infiltration of the alveoli, septums and bronchi with a variety of cells, among which eosinophils were particularly conspicuous.

Experimentally, several authors have produced allergic pneumonia in animals. The earlier literature on attempts to produce asthma in guinea pigs has been summarized by Alexander, Becke and Holmes.[14] These authors sensitized guinea pigs to horse dander and egg white and then by sprayings of each of these allergens and direct intratracheal injections of the egg white they were able to produce typical asthmatic attacks in many animals with immediate death in some of them. They did not examine the lungs microscopically. Fried [15] sensitized rabbits to horse serum, after which he injected a small amount of the serum intratracheally and killed the animals at intervals of from one hour to several weeks. Microscopically there were within a few hours edema, granulocytic infiltration, atelectasis and periphlebitis. This was followed in twelve to twenty-four hours by consolidation, in which mononuclear cells and mononuclear eosinphils composed the bulk of the intra-alveolar exudate, and in forty-eight hours by definite lobar consolidation, accompanied by exudate in the pleural cavities. In four days the alveolar exudate began to organize and disappear. Some vessels showed

12. Smith, D. C. W., and Alexander, A. J.: South. M. J. **32**:267, 1939.

13. Broch, O. J.: Acta med. Scandinav. **113**:311, 1943.

14. Alexander, H. L.; Becke, W. G., and Holmes, J. A.: J. Immunol. **11**:175, 1926.

15. Fried, B. M.: J. Exper. Med. **57**:111, 1933.

a cuffing of lymphocytes, swelling and proliferation of the intima, and thrombosis. Cannon, Walsh and Marshall [16] reported an acute anaphylactic inflammation of the lungs of rabbits sensitized to egg white and crystalline egg albumin. The lesions consisted of edema, alveolitis, bronchitis, pneumonic consolidation and acute arteritis and phlebitis. Despite these reports, however, and despite the ever increasing literature on Löffler's syndrome in man, no one has attempted to follow the allergic pneumonia in animals roentgenographically and to correlate these observations with the accompanying clinical manifestations and pathologic alterations. In other words, no one has attempted to reproduce in animals the syndrome as reported by Löffler and others in man. For this reason, and to understand better the underlying pathologic changes in man which are so meagerly reported, the following experiment was undertaken.

MATERIAL AND METHOD

After several preliminary trials 13 rabbits, each weighing approximately 2,200 Gm., were sensitized to horse serum by subcutaneous abdominal injections of 0.5 cc., made at the rate of one every other day until a total of nine injections had been given. Eighteen days after the last injection 0.1 cc. of serum was introduced into the skin of the lateral portion of the abdomen several inches away from the site of the sensitizing injections. Two days later the hair over the ventral surface of the neck was clipped, and under light ether anesthesia the trachea was exposed. This procedure was also carried out on 9 normal rabbits. The rabbits were then divided into two groups. Group 1 consisted of 6 sensitized rabbits (5, 6, 7, 8, 9 and 11) and 4 controls (17, 18, 19 and 20). Each of these received a single intratracheal instillation of 5 cc. of horse serum. Group 2 consisted of 7 sensitized animals (1, 2, 4, 10, 12, 94 and 95) and 5 controls (13, 14, 15, 16 and 66). Each of these received a preliminary intratracheal instillation of 0.5 cc. of horse serum and subsequent instillations of a similar amount at hourly intervals until a total of from 5 to 12 doses had been received (five on the first day and the remainder on the second day). Three rabbits (94, 95 and 66) received an additional intratracheal instillation of 1 cc. on the fourth day. The cervical wound in each animal was closed when the allotted instillations were completed. All rabbits received the initial instillations while they were still partially anesthetized. The rectal temperature, a differential blood smear and a roentgenogram of the thorax had been recorded before the trachea was exposed. Roentgenograms were taken again twenty-four hours after the single instillation in group 1 and three hours after the multiple instillations had been completed on the second day in group 2. Subsequently roentgen examinations were made at intervals of one and several days depending on the progress of the pneumonia, and of all animals at the time of death. Blood smears were made and rectal temperatures were recorded to accompany the roentgenograms until it was certain that they were stabilized. All roentgenograms were made with the rabbits in an erect position, stretched out on a specially constructed board, and a small focal spot rotating anode tube was used, with 300 milliamperes of current and 70 kilovolts at 1/120 second and a distance of 36 inches (91.5 cm.). The animals died or were killed (by an

16. Cannon, P. R.; Walsh, T. E., and Marshall, C. E.: Am. J. Path. **17**:777, 1941.

intravenous injection of ether) at intervals of from twelve hours to fifteen days and the trachea and the lungs were examined grossly and microscopically. While still in situ the trachea was carefully opened and a smear made of its secretions. This was stained for eosinophils by applying first Wright's stain in the usual manner, decolorizing in 70 per cent alcohol and counterstaining with methylene blue. At least 300 leukocytes were counted, and only those with unmistakably large eosinophilic granules were considered as eosinophils. In addition to the tracheas and lungs of the aforementioned 22 animals we studied microscopically those of several normal untreated rabbits and those of others treated exactly like the rabbits in group 2 except that the lesions were not followed roentgenographically.

RESULTS

Clinical Manifestations.—All sensitized animals reacted positively to the intracutaneous injection of horse serum. Within five hours after the injection an area of erythema and edema appeared that measured as much as 5 cm. across and 1.2 cm. in depth. In twenty-four hours the area of erythema increased to as much as 7 cm. in diameter, with a central dark region of beginning necrosis appearing in it in some of the rabbits. The reaction reached its maximum intensity in forty-eight hours, after which the erythema and the edema subsided and the necrosis sloughed. The control rabbits in group 1 showed no untoward signs following the intratracheal instillation of the serum. In all the sensitized animals in this group a few rales developed, which cleared in several hours, but only in 1 did anaphylaxis develop. About two minutes after receiving an injection rabbit 5 began to cough, sneeze, shake his head, thresh around and breathe rapidly, so that for about thirty seconds it appeared as though he would die. He promptly recovered, however, and remained visibly well thereafter. There were no severe reactions in the sensitized rabbits of group 2. Some of the animals, particularly 10, 2 and 1, showed evidence of obstruction subsequent to the second and third instillations. Inspiratory dyspnea was unmistakable and became increasingly severe with each instillation. Rabbit 10 was found dead in the cage five hours after the last instillation on the first day and rabbits 2 and 1 became extremely cyanotic and dyspneic after the first and the second instillation, respectively, on the second day. Each ceased to breathe a few minutes later. In both these and the remaining sensitized rabbits rales and snorting developed after the initial intratracheal instillations. The former disappeared shortly after the instillations were stopped, but the latter persisted for several days. Both rales and snorting were also present in 2 of the control animals of group 2 but were not nearly as apparent as they were in the sensitized rabbits, nor did they last as long. None of the controls showed cyanosis or inspiratory dyspnea.

There was no significant alteration of the temperature of any of the animals. The highest recorded rise was 2 F., but in some there was a drop of 2 F. below the previous normal, and the changes were similar in all the animals. Changes in the numbers of eosinophils in the blood smears were likewise insignificant. Three sensitized rabbits (5, 7 and 9), whose normal eosinophils constituted 3, 8 and 0 per cent of the total leukocyte count, showed an increase to 11, 23 and 16 per cent, respectively, but these were counterbalanced by an increase in control animals (14 and 16) from 2 to 31 per cent and 1 to 11 per cent respectively. The remaining animals in both groups showed no noteworthy changes.

Roentgenographic Changes.—Serial roentgenograms showed the development of transitory pulmonary infiltrations that reached their maximum intensity in from twenty-four to seventy-two hours after intratracheal instillation and then cleared

rapidly in from seven to thirteen days. The distribution of the pulmonary lesions was by no means uniform. One or more lung fields were involved in single or multiple areas. The extent of the consolidation varied considerably; in some cases there was complete lobar distribution, in others dense areas radiated outward from the hilar regions, and in still others there were small, fairly discrete nodular infiltrations of the lung parenchyma. The shadows were usually dense and fairly well demarcated, although sometimes they were ill defined. None of the controls had isolated lesions peripherally in the lung fields, nor was involvement of the pleura noted (figs. 1 and 2).

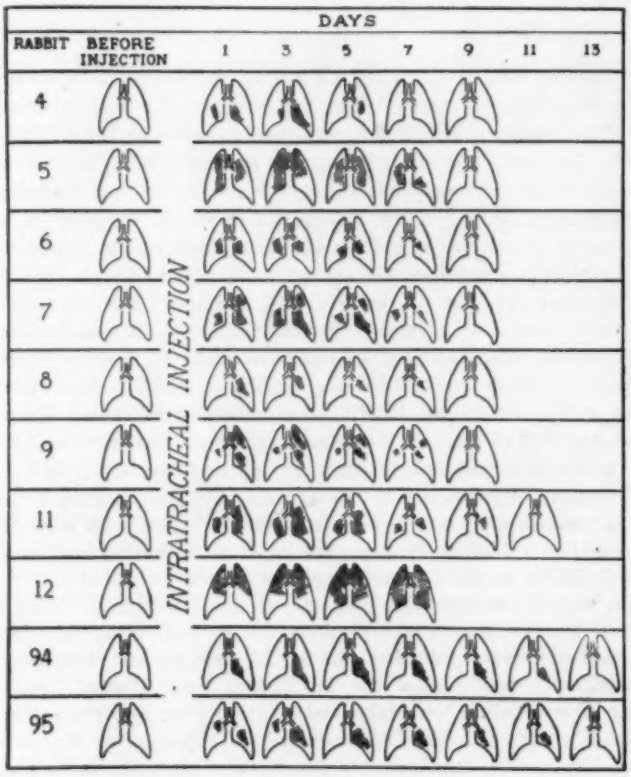

Fig. 1.—Distribution and duration of pulmonic infiltrations shown by serial roentgenograms.

More specifically, in group 1, with single instillations being made, all the sensitized rabbits (5, 6, 7, 8, 9 and 11) showed pulmonic infiltrations, which were variable in degree and distribution, as can be seen from figure 1. These infiltrations reached a maximum degree in from twenty-four (8) to forty-eight (5, 6, 7, 9 and 11) hours and showed complete clearing roentgenographically in from nine (5, 6, 7, 8 and 9) to eleven (11) days. None of the controls in this group had demonstrable pulmonary lesions. In group 2, with multiple instillations being given, all sensitized rabbits (1, 2, 4, 12, 94 and 95) had significant pulmonary infiltrations within twenty-four hours after the first intratracheal instillation. Rabbits 1 and 2 showed extensive consolidation of both upper lobes, together

with considerable involvement of the inner zones of the lower lobes in rabbit 1 and more extensive infiltration of both lower lobes in rabbit 2. These animals died twenty-four hours after the first instillation. In rabbit 12 the pulmonic process involved both upper lobes, the middle lobe of the right lung and the inner zone of the lower lobe of the left lung—all within twenty-four hours. Instead of clearing, the consolidation became progressively more dense. In forty-eight hours there was atelectasis of the middle lobe of the right lung, followed later by bilateral empyema. The animal died on the eighth day. The lesions in rabbit 4 cleared in seven days, and those in rabbits 94 and 95 cleared in thirteen days.

Pathologic Changes.—In all the sensitized rabbits of group 1 pneumonia developed that cleared roentgenographically, and at autopsy the lungs of all rabbits

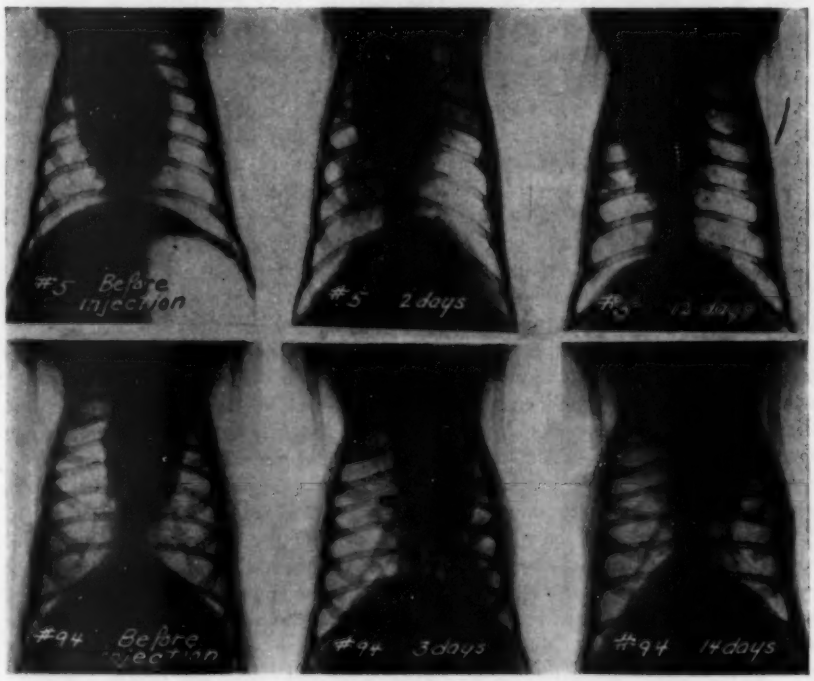

Fig. 2.—Roentgenograms of rabbits 5 and 94 taken before intratracheal instillation of serum, at the height of the pneumonic process and after clearing.

but 5 and 11 were entirely normal. The former showed a group of small abscesses of a total diameter of 6 mm. in the medial portion of the lower lobe of the left lung. The latter had 2 areas of atelectasis, each 3 mm. in diameter; one was at the tip of the lower lobe of the left lung, and the other was at the base of the upper lobe of the right lung. The roentgenograms of the controls in this group did not present shadows at any time, and at autopsy the lungs of these rabbits were normal. The peritracheal tissue was free of infection in each rabbit of this group. In group 2, 3 of the 7 sensitized rabbits (10, 2 and 1) died on the first and second days of the instillation of serum. In a fourth rabbit (12) a peritracheal abscess developed, which extended into the mediastinum and each pleural cavity, producing bilateral empyema. In the remaining 3 animals (4, 94 and 95) pneumonia

developed that cleared roentgenographically, but at postmortem examination the lungs of rabbits 4 and 95 only were clear, whereas the medial portion of the lower lobe of the left lung of rabbit 94 contained a focus of several tiny conglomerate abscesses that measured 9 by 4 mm. The roentgenograms of 3 of the controls (66, 14 and 15) in group 2 did not present shadows, but those of 2 (13 and 16) showed pulmonic infiltrations that first appeared at twenty-four hours. Each of the latter at autopsy showed a peritracheal and mediastinal abscess and bilateral empyema. Rabbit 13 disclosed in addition massive atelectasis of each upper lobe. In all animals of group 2 except 4, 94, 95, 66 and 14 a peritracheal abscess developed, and, as already stated, in rabbits 12, 13 and 16 it trekked down the mediastinum and was continuous with pleural empyema.

The lungs as examined at various intervals of time after intratracheal instillation of the serum was completed disclosed the following pathologic changes: Within twelve hours there was intense congestion of the mucosa of the larynx, the trachea and the bronchi, with no obstruction of the lumen. The lungs showed petechiae and atelectasis. Sometimes the latter was massive and occupied an entire lobe, but usually it was focal and irregularly distributed. Microscopic sections of the trachea disclosed some sloughing of the epithelial cells, intense engorgement of the submucosal capillaries and only a sprinkling of plasma cells, lymphocytes, eosinophils and polymorphonuclear leukocytes. Sections of the lungs showed extreme congestion, with edematous fluid filling most of the alveolar spaces and disrupting the alveolar walls (fig. 3 *A*). Inflammatory cells were entirely absent. Peripherally there were extensive areas of emphysema wherein the alveoli were greatly distended and their septums often irregularly ruptured. The lymphatic vessels were inconspicuous, and the arteries were normal.

At twenty-four hours the lumen of the tracheobronchial tree was still patent but filled with frothy serosanguineous fluid, and the mucosa was congested. Besides atelectasis, congestion and petechiae, the lungs disclosed irregularly shaped and distributed areas of pneumonia. The portions not involved by the aforenamed processes were voluminous (at least twice the normal size) and comparatively pale. Histologic sections of the trachea and the larger bronchi showed in addition to the previously mentioned alterations vacuolation of the superficial epithelial cells, fibrinous degeneration of the connective tissue beneath the basement membrane and diffuse infiltration with eosinophils and a few plasma cells (fig. 3 *B*). Thrombi were present in some of the submucosal vessels. The epithelium of the bronchioles was essentially the same, but in some areas it was disrupted by an intense eosinophilic infiltration. Many of the lumens were completely plugged with eosinophils or mixtures of these cells, sloughed epithelial cells and debris. Sections of the lungs revealed some areas in which only atelectasis, acute emphysema or pneumonia was present and other areas in which there was intermingling of these three processes (fig. 3 *C*). The consolidated areas were either large and diffuse or small and patchy and showed some congestion and edema but more conspicuously infiltration with eosinophils, which in some areas composed 100 per cent of the infiltrating cells. In other sections they were less conspicuous but nevertheless always present. The remaining cells were chiefly polymorphonuclear leukocytes and plasma cells. The perivascular lymphatics were distended with edematous fluid, and some arteries showed swelling and vacuolation of the endothelial cells. There was no pleuritis.

At forty-eight hours the only gross differences of the lungs as compared with those already described were that the visceral pleura was covered with a thin fibrinopurulent exudate and consolidation was more diffuse, so that sometimes an entire lobe was involved. Histologically there were still present areas of congestion,

edema, atelectasis and emphysema. In some sections the pneumonia was diffuse and of the lobar type with a uniform infiltration that was almost 100 per cent eosinophils (fig. 3 *C*). In other areas, however, the exudate was less dense and for the first time disclosed large round vacuolated phagocytes. The pleural exudate consisted of many neutrophils and fewer eosinophils intermingled with varied amounts of fibrin. Some of the arteries were cuffed with eosinophils, but none showed lesions resembling periarteritis nodosa. The perivascular lymphatics were distended with serum and conspicuous.

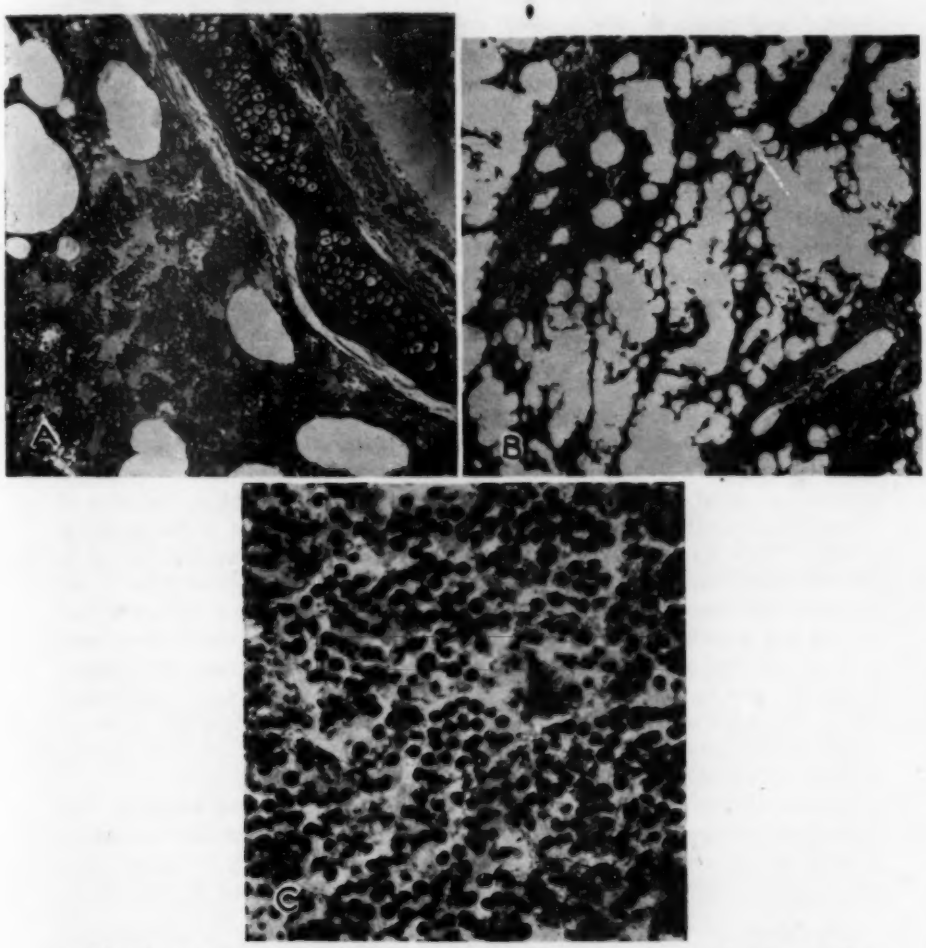

Fig. 3.—*A*, section of a bronchus and adjoining lung made twelve hours after intratracheal instillation of serum was completed, showing congestion of the alveoli and edematous fluid in the alveolar spaces and the bronchial lumen. Hematoxylin and eosin; × 75.

B, section of a lung made twenty-four hours after instillation of serum was completed, showing areas of atelectasis and pneumonia alternating with areas of acute emphysema. Hematoxylin and eosin; × 37.5.

C, section of a diffusely consolidated lung made forty-eight hours after instillation of serum was completed, showing faint alveoli in the background and a dense infiltrate of eosinophils. Hematoxylin and eosin; × 400.

The lungs examined at seventy-two hours were essentially the same in gross appearance as those examined at forty-eight hours. Microscopic sections of the trachea showed normal mucosa and submucosa. In some areas the lungs were similar microscopically to those examined at forty-eight hours, but in others they revealed the following differences: The septums were thick, prominent and cellular; the exudate was heavier, contained more fibrin and was well on the way to organization, and large intra-alveolar phagocytes were particularly conspicuous. Because we did not have as many animals as we wished, none was killed after seventy-two hours until complications arose or until roentgenograms showed normal lung shadows. We assumed, therefore, that there was a continuation of organization and resolution along the lines seen at the seventy-two hour interval in the rabbits in which a pneumonic process developed that cleared, with the lungs appearing normal at autopsy.

Three significant complications developed in a few rabbits, namely, (1) residual focal pulmonary abscesses in 3 sensitized animals (12, 94 and 5), (2) empyema in 1 sensitized rabbit (12) and 2 controls (13 and 16) and (3) peritracheal abscesses in one half of the rabbits receiving multiple instillations. Histologic sections of the earliest abscesses disclosed central areas of debris and nuclear fragments, sharply demarcated from the immediately surrounding collapsed alveoli, which contained phagocytes, and beginning proliferation of fibrous tissue. The older abscesses showed calcific deposits in the central areas, around which were a few spurious giant cells. Peripheral to this were nuclear fragments intermingled with few neutrophils and phagocytes. Beyond these there was a zone of granulation tissue consisting of a background of young fibroblasts rich in capillaries and diffusely infiltrated by eosinophils and scattered neutrophils, lymphocytes, plasma cells and phagocytes. These pulmonic abscesses were in every microscopic detail duplicates of the cutaneous abscesses formed at the sites of intradermal inoculation and of the peritracheal abscesses formed in the sensitized rabbits. They differed from those formed in the necks of unsensitized rabbits in that the latter contained few or no eosinophils. The septums about some of the abscesses and particularly those beneath the pleura showed some thickening and fibrosis and were infiltrated with plasma cells and lymphocytes. Some were lined with tall cuboidal cells identical with those lining the bronchioles, and their lumens contained many vacuolated phagocytes mixed with a few plasma cells and lymphocytes. Histologic sections of the pleura in each of the 3 rabbits in which empyema developed disclosed a mixture of neutrophils, plasma cells and other monocytes, granular debris and some fibrin. All were devoid of eosinophils.

Eleven of the 22 rabbits showed eosinophils in the tracheal secretion. Of these, 3 were control rabbits 13, 66 and 15, disclosing 1, 2 and 5 per cent eosinophils, respectively, whereas the remaining 8 were sensitized rabbits, 95, 10, 7, 6, 1, 2, 9 and 5, which disclosed, respectively, 7, 12, 20, 21, 30, 64, 67 and 81 eosinophils.

COMMENT

Although it is difficult to evaluate clinical manifestations in the rabbit and particularly to correlate them with those in man, several more obvious observations do nevertheless appear to be in order. All authors have repeatedly stressed scarcity of signs and symptoms of the condition known as Löffler's syndrome as it develops in man. This dearth of clinical manifestations has also been noted in the rabbits in which the

lesions were produced experimentally. Anaphylaxis following the first intratracheal instillation of serum was probably avoided in all our animals except 1 by giving the instillation while the anesthesia was still effective. In rabbit 5 coughing, sneezing, threshing about and rapid breathing were observed a few minutes after the animal received the serum, but in the remaining sensitized animals in group 1 only a few rales developed, which cleared in several hours. In group 2 (which received multiple instillations) inspiratory dyspnea became manifest in several of the sensitized animals and snorting in most of them. The former was obviously not a sign of bronchial asthma, because in this condition the dyspnea is expiratory. At autopsy none of the rabbits showed obstruction of the larynx or the trachea, but the lumen of the tracheobronchial tree was filled with edematous fluid and the smaller bronchi and bronchioles were plugged with desquamated epithelium and cells of inflammatory origin. These changes appeared to be sufficient to account for the dyspnea. There was no evidence of spasm of the bronchi or the bronchioles. Snorting, unless it was a manifestation of dyspnea, could not be accounted for. The rales that appeared in the sensitized animals of group 2 were only transitory and disappeared shortly after the instillations were stopped. In all instances they were probably caused by the instilled serum plus the edematous fluid, which microscopically was conspicuous.

Another similarity between the syndrome occurring in man and that produced in rabbits is the lack of disturbance of the temperature. In man, if fever is present, it is slight. In none of the animals did the reading differ more than 2 F. from the previous normal, and the temperature changes in the control rabbits were similar to those in the sensitized. Although the eosinophilic count of the sputum of man has been infrequently recorded, there are indications that it is often increased.[17] There was an unequivocal increase in that of rabbits. Of the 11 animals that showed eosinophils in smears of tracheal secretions, only 3 were controls, disclosing counts of 1 to 5 per cent, whereas 8 were sensitized rabbits, which revealed counts of 7 to 81 per cent. The same parallelism, however, did not hold regarding the eosinophils of the blood stream. In listing the criteria for a diagnosis of the syndrome in man almost all authors include eosinophilia. Soderling,[8a] however, stated that too much significance should not be attached to an increase of eosinophils in the blood, for such cells may be temporarily or entirely absent. Cases without eosinophilia, albeit in the minority, have been reported.[18] The rabbits' blood smears showed no appreciable alteration

17. Löffler.[1] Harkavy.[9b] Kartagener.[11]

18. Beck.[3e] Peabody.[2b]

of the eosinophilic count. Thus, in summing up the clinical aspects one may say that the constitutional disturbances in the syndrome as produced in rabbits were minimal, and so paralleled closely those seen in man.

There was also a close correlation between the roentgenograms of man and those of the rabbits in which the lesions were produced experimentally. Löffler [1] described the former as showing homogeneous or, more often, spotty or cloudlike, sharply or less sharply limited, single or multiple, unilateral or bilateral, migratory shadows that appeared and disappeared in from three to eight days. Occasionally they also showed localized areas of pleural involvement and small pockets of loculated fluid. Depending on the distribution of the shadows, he divided his cases into the following five groups: (1) those in which the roentgenograms presented large, more or less irregular unilateral or bilateral shadows, (2) those in which the shadows represented small round foci, (3) those in which multiple lesions were shown in one or both lungs, (4) those in which the lesions were sharply limited to a lobe and (5) those in which the lesions resembled tuberculous infiltrations. In the roentgenograms of the rabbits the shadows were for the most part confluent and spreading outward from the hilus. Some, however, were spotty and nodular and were seen in the inner zones of the lung fields. There were no rounded foci at the periphery, nor were there visible involvements of the pleura or collections of fluid except in those few animals in which empyema developed. The chief difference between the shadows of man and those of rabbits was the lack of migration of the latter. In animals the areas of infiltration developed to a maximum and then cleared without reappearing or shifting to other portions of the lung fields. The reason for this difference may lie in the fact that in man the allergen is present over a prolonged period, whereas in all but 2 experimental animals the lungs were not exposed to the allergen after the initial instillation or series of instillations was completed.

The pathologic changes observed in the lungs of rabbits followed closely those seen in man in the few recorded instances in which the human patient died of the syndrome.[19] In the latter the changes consisted of: hyalinization of the basement membrane of the bronchial epithelium, together with edema and eosinophilic infiltration of the submucosa; infiltration of the alveolar walls with eosinophils, neutrophils and lymphocytes; thrombosis of small vessels, and, in the alveoli, congestion, edema, atelectasis and emphysema. In rabbits the only difference in the walls of the trachea and the bronchi was absence of hyalinization of

19. Harkavy.[8c] Broch.[13]

the basement membrane. In the lung proper the changes consisted of congestion, edema, atelectasis, emphysema and eosinophilic pneumonia.

As a rule, the lesions in man clear completely, leaving no residuum roentgenographically. This was also true in all animals of group 1 and in most of the surviving ones of group 2. Pathologically, however, 3 of these rabbits disclosed single groups of conglomerate abscesses. In 2 animals the abscesses were located in the medial portion of the lower lobe of the left lung and were obscured by the shadow of the heart, and in a third they were obscured by empyema. That these abscesses were the remains of allergic pneumonia is attested by their microscopic similarity to the lesions produced at the sites of intradermal injection. If the rabbits had lived longer, even these foci might have resolved completely or become fibrosed. One other animal (11) in which the lesions cleared roentgenographically disclosed at autopsy two tiny residual areas of atelectasis. There is, of course, no way of telling whether such complications do or do not sometimes follow the syndrome in man. The formation of peritracheal abscesses in group 2 was undoubtedly the result of direct external contamination in all animals together with a specific allergic response to extravasated serum in the sensitized ones. The latter view is borne out by the fact that eosinophils were present in the abscess walls of the sensitized rabbits and were not present in those of the controls. Because empyema was observed only in those rabbits that showed peritracheal and mediastinal abscesses it was considered as a direct extension of the infection of the neck and the mediastinum and not as a result of the pneumonitis. Empyema therefore has no part in the syndrome and in our experiment was an accidental complication in 3 rabbits.

SUMMARY

From a review of the literature it is apparent that most of the knowledge of the pathologic changes occurring in the lungs of man in Löffler's syndrome has been speculative. In two acceptable reports the lesions are listed as hyalinization of the basement membrane of the mucosa of the bronchi together with edema and eosinophilic infiltration of the submucosa, and congestion, edema, atelectasis, emphysema and eosinophilic pneumonia of the lung proper.

In the present experiment we have succeeded in duplicating in rabbits the syndrome as observed in man. Sensitized animals were given single or multiple intratracheal instillations of horse serum. They presented only slight clinical disturbances; roentgenographically they revealed transitory pulmonic infiltrations that cleared in from seven to thirteen days; there were eosinophils in the tracheal secretions, and pathologically there were congestion, edema and eosinophilic infiltration of the sub-

mucosa of the trachea and the bronchi, and in the lung parenchyma there were congestion, edema, atelectasis, emphysema and an eosinophilic pneumonia. The syndrome in rabbits differed from that usually seen in man in that (1) there was no eosinophilia and (2) the pulmonic shadows were not migratory.

It is concluded that Löffler's syndrome is an allergic inflammation of the lungs and that one route by which the allergen invades is that of inhalation.

ADRENAL HEMORRHAGES IN MENINGOCOCCIC SEPSIS

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A FULMINANT type of sepsis, with purpura and cyanosis of the skin and adrenal hemorrhages, frequently occurring in the presence of a thymolymphatic constitution (especially in children) and generally caused by Neisseria meningitidis (Neisseria intracellularis), is wrongly given the name "Waterhouse-Friderichsen syndrome."

Neither Waterhouse [1] nor Friderichsen [2] was the first to describe this syndrome, but Voelcker,[3] in 1894. Graham-Little [4] compiled all previously published cases in 1901, and only after several other authors. Waterhouse [1] in 1911 and Friderichsen [2] in 1918 published studies, whose merit does not justify the use of the name "Waterhouse-Friderichsen syndrome" suggested by Thomas [5] and later by Glanzmann [6] and others.[7]

It seems that a descriptive term such as "fulminant sepsis with adrenal hemorrhage" (Schwarz [7a]) or "the meningococcic adrenal syndrome" (Banks and McCartney [7b]; Holmes and Cowan [8]) would be more appropriate and less erroneous than the present name.

The bacterium most frequently found in cases of the syndrome is Neisseria meningitidis and the presence of other bacteria described by some authors could not have been verified by modern methods. It seems

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1. Waterhouse, R.: Lancet **1**:577, 1911.

2. Friderichsen, C.: Jahrb. f. Kinderh. **87**:109, 1918.

3. Voelcker, A. F.: Pathological Reports of the Middlesex Hospital, London, 1894, p. 279.

4. Graham-Little, E.: Brit. J. Dermat. **13**:445, 1901

5. Thomas, E., in von Pfaundler, M., and Schlossmann, A.: The Diseases of Children, translated by M. G. Peterman, Philadelphia, J. B. Lippincott Company, 1935, vol. 2.

6. Glanzmann, E.: Jahrb. f. Kinderh. **139**:49, 1933.

7. (*a*) Aegerter, E. E.: J. A. M. A. **106**:1715, 1936. (*b*) Banks, H. S., and McCartney, J. E.: Lancet **1**:771, 1943. (*c*) Herbut, P. A., and Manges, W. E.: Arch. Path. **36**:413, 1943. (*d*) Lindsay, J. W., and others: Am. J. M. Sc. **201**: 263, 1941. (*e*) Rabinowitz, M.: ibid. **166**:513, 1923. (*f*) Sacks, M. S.: Ann. Int. Med. **10**:1105, 1937. (*g*) Schwarz, J.: Rev. sud-am. morfol. **2**:69, 1944. (*h*) Thomas, H. B., and Leiphart, C. D.: J. A. M. A. **125**:884, 1944.

8. Holmes, J. M., and Cowan, J. M.: Lancet **1**:13, 1945.

that Andrewes,[9] in 1906, was the first to announce the meningococcus as the agent causing the syndrome, but this achievement is attributed by most authors to MacLagan and Cooke [10] in spite of a gap of ten years between both publications.

Descriptions of the intracranial anatomic lesions vary from "none" to presence of purulent meningitis or encephalitis (Banks and McCartney [11]), but in several cases in which macroscopically there were no lesions microscopic meningitis has been demonstrated (Schwarz [7g]); therefore, in cases in which clinically there are no meningeal signs and in which macroscopic inspection of the leptomeninx appears to yield negative results there may be definitive inflammation of the latter.

A large thymus and hyperplastic lymph nodes and follicles were observed by numerous authors, notably by Baumann,[12a] Ghon,[12b] Mitchell and Rittershofer [12c] Aegerter,[7a] Herbut and Manges,[7c] Rabinowitz,[7e] Schwarz and co-workers,[13] Martland [14] and recently by Holmes and Cowan,[8] who reported the case of an 8 year old girl with a thymus of the enormous weight of 41 Gm. The humoral chemism of the syndrome is not completely known yet, because of the swift development of the disease (six to forty-eight hours).

During the years 1941 to 1944 there was an epidemic of meningococcic meningitis in Chile. Not less than 9,669 cases were reported to the National Health Service; it may be supposed that the real number was much larger. Fulminant sepsis with adrenal hemorrhages was observed in more than 100 children, being especially prevalent during 1942 and 1943, and in practically all it came to a lethal end (Meneghello [15]; Steeger [16]; Simpfendorfer [17]).

The present study concerns the histologic appearance of the adrenal glands in 5 infantile cases (table).

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9. Andrewes, F. W.: Lancet **1**:1172, 1906.

10. MacLagan, P. W., and Cooke, W. E.: Lancet **2**:1054, 1916.

11. Banks, H. S., and McCartney, J. E.: Lancet **1**:219, 1942.

12. (*a*) Baumann, T.: Ztschr. f. Kinderh. **51**:276, 1931. (*b*) Ghon, A.: Med. Klin. **30**:695, 1934. (*c*) Mitchell, G., and Rittershofer, C. R.: The Thymus Gland, in Brennemann, J.: Practice of Pediatrics, Hagerstown, Md., W. F. Prior Company, Inc., 1944, vol. 3, chap. 22.

13. Meneghello, J.; Schwarz, J., and Steeger, A.: Rev. chilena de pediat. **15**:1010, 1944. Schwarz.[7g]

14. Martland, H. S.: Arch. Path. **37**:147, 1944.

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16. Steeger, A., and Banfi, E.: Rev. méd. de Chile **71**:426, 1943.

17. Simpfendorfer, E.: Rev. chilena de pediat. **15**:1027, 1944.

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF THE ADRENAL GLANDS

CASE 1.—Both adrenal glands contained hemorrhagic zones. Microscopically, the glands, which were narrow had numerous partially coalescing hemorrhagic foci in the medulla and intense hyperemia of the cortex, with extreme dilatation of the capillaries, especially those in the two inner layers of the cortex. The capillary dilatation was intense enough to compress and flatten the cortical tissue. No difference was noted between the left and the right gland. No inflammatory lesions were seen.

CASE 2.—The left adrenal gland seemed normal; the right gland presented intense hyperemia in its lower two thirds.

Microscopically, the left gland showed intense hyperemia of the medulla with several small hemorrhages. The right gland showed intense hyperemia with

Summary of Observations in Five Cases of Fulminant Meningococcic Sepsis

Case; Sex; Age of Patient	Duration of Life After Onset of Illness, Hr.	Meninges		Weight of Thymus, Gm.	Weight of Brain, Gm.	Skin		Cerebrospinal Fluid	Neisseria Meningitidis	Kernig's Sign
		Macroscopic	Microscopic			Cyanosis	Purpura			
1, M 10 yr.	20	Opalescent	Meningitis	45	1,350	+++	+++	?	?	+
2, M 2 mo.	29	Purulent	Meningitis	35	?	+	+	Purulent	+	0
3, F 2 mo.	9	Clear	Meningitis	30	570	+++	+	?	+	0
4, F 1 yr.	15	Clear	Meningitis	38	1,120	+++	+++	Normal	0	0
5, F 3 mo.	15	Clear	Meningitis	15	660	+++	+++	Normal	0	0

circumscribed hemorrhages which were partially confluent in the medulla. The structure of the cortical zone was completely conserved. There was not even hyperemia. No inflammatory lesions were seen. The thickness of the glands was greatly reduced.

CASE 3.—The adrenal glands were completely destroyed by hemorrhages.

Microscopically, the lesions were almost the same in both glands. The thickness of the glands was much reduced. Both medullas were completely destroyed by hemorrhages. The cortices presented intense hyperemia in the lower layers and also small hemorrhages. There were no inflammatory lesions.

CASE 4.—The adrenal glands were completely destroyed by hemorrhages.

Microscopically, the lesions were almost the same in both glands. Each gland was narrow, with extremely intense hyperemia in the cortex, so that cortical tissue could not be seen, and it was impossible to distinguish whether red blood cells were in or outside the capillary walls. In the zona fasciculata there were several necrotic foci. The medulla had changed into a pool of blood. No inflammatory lesions were seen.

CASE 5.—Each adrenal gland showed hemorrhages, especially in the medulla. Microscopically, the glands were broad, with extraordinary hyperemia through the cortex and the medulla and multiple hemorrhages confluent in the medulla, more isolated in the cortical layers. Practically all tissue of both glands had been destroyed.

The examination of these adrenal glands revealed as a common characteristic of such cases hypoplasia of the adrenal glands (cases 1 to 4). In no case was thrombosis observed—neither in large veins nor in minor blood vessels—and the tables referring to this point published by Banks and McCartney [7b] are unconvincing. Neither have inflam-

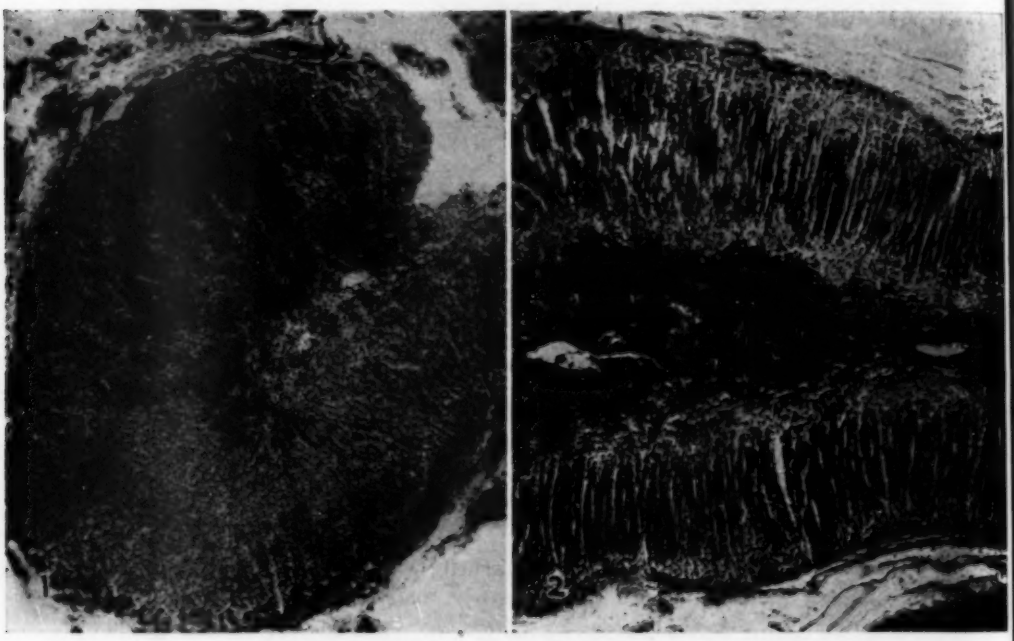

Fig. 1.—Intense dilatation of cortical capillaries with hemorrhage by diapedesis. No diffuse destruction is seen in this region, but illness was acute, lasting in all twenty hours. Hematoxylin-eosin; × 23.

Fig. 2.—Right adrenal gland showing diffuse destruction of the medulla by hemorrhage. Note the normal structure of the cortical zone. The total duration of illness was twenty-nine hours. Hematoxylin-eosin; × 23.

matory foci, described by the same authors, been observed in these 5 cases. Apparently there exist great differences in this matter. In my experience, lesions have been more serious in, and sometimes limited to, the medulla; this makes one suppose that lesions appear first in this part of the organ and later extend to the cortex. Usually cortical lesions are observed to be much less destructive than lesions of the medulla.

The macroscopic and microscopic appearances of the adrenal glands in these cases are impressive, but if one studies hormonal relations and realizes that the cortex, not the medulla, is indispensable for human life, one cannot feel satisfied with the simple explanation held forth in many papers on the subject, that in such cases sudden death is due to destruction of the adrenal glands (Ghon [12b]; Baumann [12a]; Levinson [18]). It is known that the cortex, not the medulla, is essential to human life, because substances similar to epinephrine can be produced in other regions. On the other hand, it is known that the medulla is a storehouse of the body's supply of epinephrine, and bleeding into the medulla might suddenly cause the entire stored amount of epinephrine to enter the circulation. In animals the injection of a large dose of epinephrine causes a short increase of pressure followed by severe and long hypotension (Grollmann [19]). Besides, there are relations between the thymus and the adrenal glands. Anatomically it may be stated that hyperplasia of the thymus is accompanied by hypoplasia of the adrenal glands. At all autopsies in the cases which I have observed there was marked hyperplasia of the thymus, and at the same time in the first 4 cases the adrenal glands were narrow. Maybe it is a coincidence but at any rate it is worthy of notice that in case 5, in which the weight of the thymus was lowest (15 Gm.), the thickness of the adrenal glands had not been reduced.

In none of these cases was it possible to weigh the gland as an index to its real volume, because the increase caused by hemorrhage would have completely falsified the result. Besides, in these cases the suprarenal glands were hard and dark red, and the surface of the incision was dry; it was impossible to squeeze out blood. This differs from the description of adrenal hemorrhage in the newborn, in which instead of the adrenal gland it is usual to find a "sac" of hemorrhagic contents.

COMMENT

Holmes and Cowan [8] observed in 3 cases edema of interstitial tissue, and they expressed the belief that death may be caused by circulatory collapse due to a serious decrease of the volume of blood. Martland [14] saw the principal cause of death in meningococcemia and not in destruction of the adrenal glands, but he did not make clear his opinion of the causes of the difference in clinical appearance and anatomic

18. Levinson, S. A.: J. Pediat. **14**:506, 1939.

19. Grollmann, A.: The Adrenals, Baltimore, Williams & Wilkins Company, 1936.

findings between cases of fulminant and cases of ordinary meningococcemia. The principal difficulty in the way of a logical explanation seems to be the question why just the adrenal vascular system should be so much affected in cases of the fulminant type; one might think of the predisposition of a pathologic gland in an organism of thymolymphatic constitution. Another factor could also influence adrenal localization: It is known that during the first months of life a physiologic transformation of the adrenal gland takes place (Dietrich and Siegmund [20]), and it is exactly in babies that the fulminant form of meningococcic sepsis is most frequently seen.

I believe that the fulminant septicemic syndrome with adrenal hemorrhages really forms a pathogenic entity. It is a serious infectious syndrome generally caused by N. meningitidis in a predisposed organism. There may be a predisposition in the thymolymphatic constitution combined with adrenal hypoplasia, which is also manifested in the pasty appearance of the children. (I can make statements about children only, as I have had no experience with adults.) Generally these children have little resistance against many and heterogenous agents (anesthetics, accidents, transfusions and punctures for example), and sudden death of these children is nothing new (Martland [14]).

If to this diminished resistance are added serious meningococcic infection and adrenal hemorrhages, a fatal ending cannot surprise one. Maybe the adrenal hyperemia—so frequently observed in autopsies during epidemic meningitis—can produce in a hypoplastic gland hemorrhages the intensity of which does not influence the clinical syndrome; in reality I can agree with Williams [21] and Thomas and Leiphart [7b] when they say that the clinical appearance is as serious in cases of unilateral or partial destruction of the adrenal glands as in cases of complete and bilateral destruction.

Naturally, therapy would be directed first against infection and secondary against possible circulatory failure due to adrenal destruction. This is based on the fact that in the experiment a complete lack of adrenal glands does not produce acute clinical symptoms during several days, so that lack of cortical hormone or of epinephrine is not the cause of this serious disease. Maybe it is a great quantity of epinephrine suddenly expelled into the blood stream: in that case, injections of epinephrine would surely not be indicated. The future will show whether this hypothesis is correct, whether in some cases hyperadrenalinemia could be established.

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SUMMARY

Adrenal hemorrhages were observed in 5 cases of fulminant meningococcic sepsis; the hemorrhages began in the medulla and occurred by diapedesis. Generally medullary lesions were much more extensive than cortical ones.

In the cases observed inflammatory lesions were not found in the adrenal glands, nor was thrombosis of the adrenal veins found. Definite hyperplasia of the thymus and hypoplasia of the adrenal glands occurred, which is interpreted as explained by the seriousness of the syndrome.

For historical reasons, the name "Waterhouse-Friderichsen syndrome" is not justified.

RADIOIODINE AUTOGRAPHY IN STUDIES OF HUMAN GOITROUS THYROID GLANDS

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THE EFFECT of radioactive iodine on the photographic plate has made it possible to trace the deposition of the radioactive element in the thyroid gland. Moreover, this disposition may then be correlated with the histologic structure of the gland.[1] Employing the radioautographic technic,* Leblond demonstrated that in rats most of the radioactive iodine, administered in varying doses,[2] was deposited in the colloid rather than in the cells [3] during periods of observation lasting thirty minutes to five days after intravenous injection of the iodine. A similar preferential distribution was shown in pituitary-treated and in hypophysectomized animals.[4] Whatever the condition of the thyroid gland, the radioiodine was more abundant in the acidophilic colloid than in the basophilic colloid,[5] perhaps owing to a more rapid turnover of iodine in the latter.[4] Following the injection of a single large dose of iodine (1 mg.), when the iodide ion was present as such in the rat's gland,[6] the autographs showed diffuse pictures even after freezing-drying fixation. This suggested that either the iodide ion was displaced during the paraffin embedding or that the picture obtained was truly representative of the diffuse distribution of the iodide throughout the epithelium and the colloid.[3a]

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3. Leblond, C. P.: (*a*) Stain Technol. **18**:159, 1943; (*b*) footnote 2.

4. Leblond, C. P.: Anat. Rec. **88**:285, 1944.

5. Footnotes 2 and 3.

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By the autographic method, Hamilton, Soley, Reilly and Eichorn [7] were able to study the deposition of radioactive iodine in human thyroid glands. They found that in goitrous glands the element was in general concentrated in the most actively functioning parts of the glands.[7a,c] In normal thyroid tissue the radioactive iodine was evenly distributed throughout the section. In nontoxic goiter [7a,c] and in goiter accompanied by severe hypothyroidism [7b] the new iodine was accumulated in the cellular portions of the gland but not in the colloid. On the other hand, in hyperplastic thyroid tissue the colloid was found to have a higher proportion of accumulated radioactive iodine than the cells of the adjacent acini, and this was explained as due apparently to the rapid outflow of this material from the hyperplastic cells.[7a,c] Cancer tissue was found to have no apparent ability to store administered radioactive iodine.[7a,c]

While the metabolism of radioiodine was being investigated in human thyroid glands,[8] removed at operation, autographic studies were undertaken to correlate, if possible, the thyroid responses revealed by chemical analyses to the distribution of iodine in the thyroid glands. Likewise, the making of radioautographs of human thyroid glands, with Leblond's technic,[3] was instituted to determine whether the administered radioiodine was to be found essentially in the colloid, as noted by Leblond in animal glands at varying intervals of time [3] and by Hamilton and his group in human hyperplastic thyroid tissue,[7a,c] or essentially in the cells, as observed by Hamilton in nontoxic goiters with [7a,c] or without [7b] accompanying hypothyroidism.

METHODS

Radioiodine containing 25 to 1,000 microcuries of the radioelement I^{131} and about 2 micrograms of iodine, I^{127}, was administered orally to R. D. and E. S. fifteen and twenty hours, respectively, before thyroidectomy. Since the radioactive iodine was administered by mouth, this dose might be considered as a physiologic amount of iodine. Autographic studies were carried out with blocks of the extirpated goiters. Bouin and Susa fluids were used for fixation. The embedding was usually done with paraffin, although in 1 case satisfactory results were obtained by mounting a whole lobe of the gland in celloidin (a concentrated preparation of pyroxylin). The various technics were speeded up by using small amounts of tissue whenever possible. When a whole lobe was included in celloidin, sections could be obtained in six days by combined use of a vacuum during clearing and the heat of a 37 degree oven during the infiltration with celloidin.

7. (*a*) Hamilton, J. G.: Radiology **39**:541, 1942. (*b*) Hamilton, J. G.; Soley, M. H.; Reilly, W. A., and Eichorn, K. B.: Am. J. Dis. Child. **66**:495, 1943. (*c*) Hamilton, Soley and Eichorn.[1]

8. Leblond, C. P.; Puppel, I. D.; Riley, E.; Radike, M., and Curtis, G. M.: Radio-Iodine and Iodine Fractionation Studies of Human Goitrous Thyroids, J. Biol. Chem., to be published.

The cut sections were affixed to the slide with the help of a 0.5 per cent solution of celloidin, and the slides were then placed in 70 per cent alcohol for a few minutes and dried. The dry slides bearing the sections were placed on the sensitive face of photographic plates (Eastman Kodak contrast lantern slide plate) in the dark room and left there for about two to three weeks. At the end of this period the plates were developed in fine grain developer and fixed as usual. The histologic slides were stained either by Masson's trichrome technic or by the hematoxylin-eosin method. The reactions on the plates were compared with

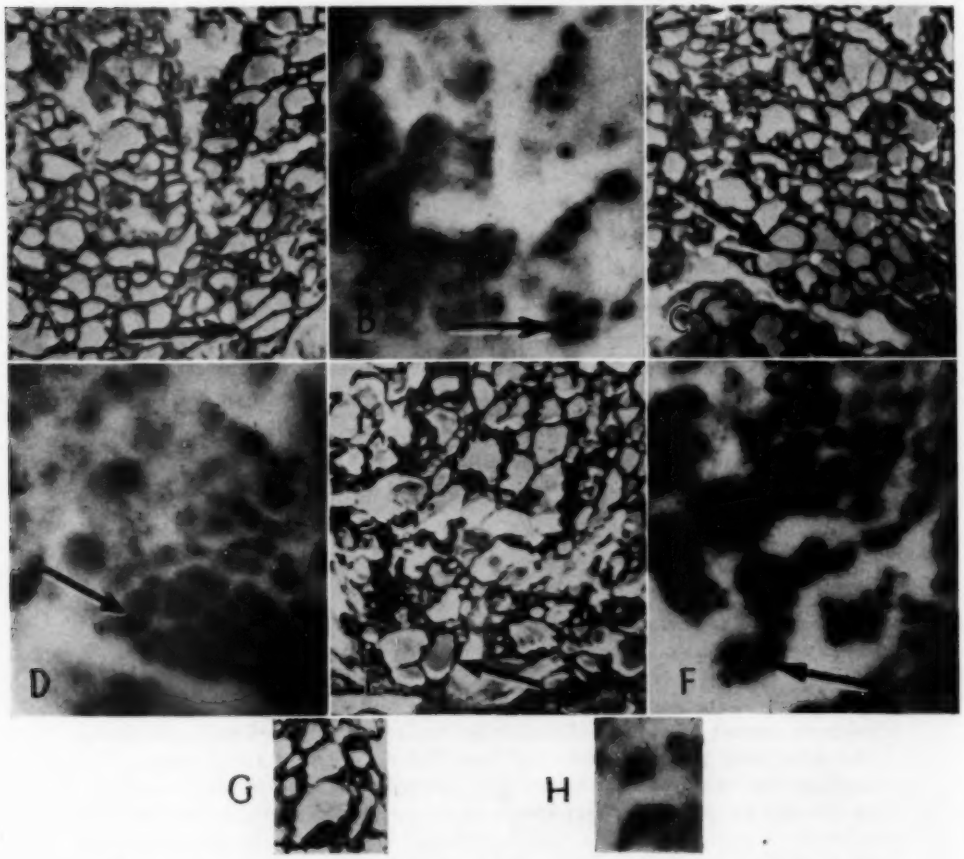

Fig. 1.—Sections of thyroid gland of a patient (E. S.) with nontoxic diffuse colloid goiter and a basal metabolic rate of minus 7 per cent, and the corresponding radioiodine autographs. A comparison of the photomicrographs (*A*, *C*, *E* and *G*) and the radioautographs of these sections (*B*, *D*, *F* and *H*) demonstrates that the radioiodine is deposited in the colloid. This remarkable feature is reemphasized when the arrow-indicated follicles of *A*, *C* and *E* are compared with the arrow-indicated radioautographic areas of *B*, *D* and *F*. Note that the radioautographic areas are almost similar in configuration to the colloid areas present. Note also that the vacuole in section *G* shows practically no radioactivity in *H*.

the histologic slides with the help of a dissecting microscope. Photographs were made of the plates (autographs) and slides at the same magnification.

It was soon found that very large tracer doses of radioactive iodine must be used to obtain satisfactory autographs from human thyroid glands. Only after administration of about 500 microcuries or more was it possible to obtain autographs sufficiently intense to lend themselves to profitable study. The autographs shown

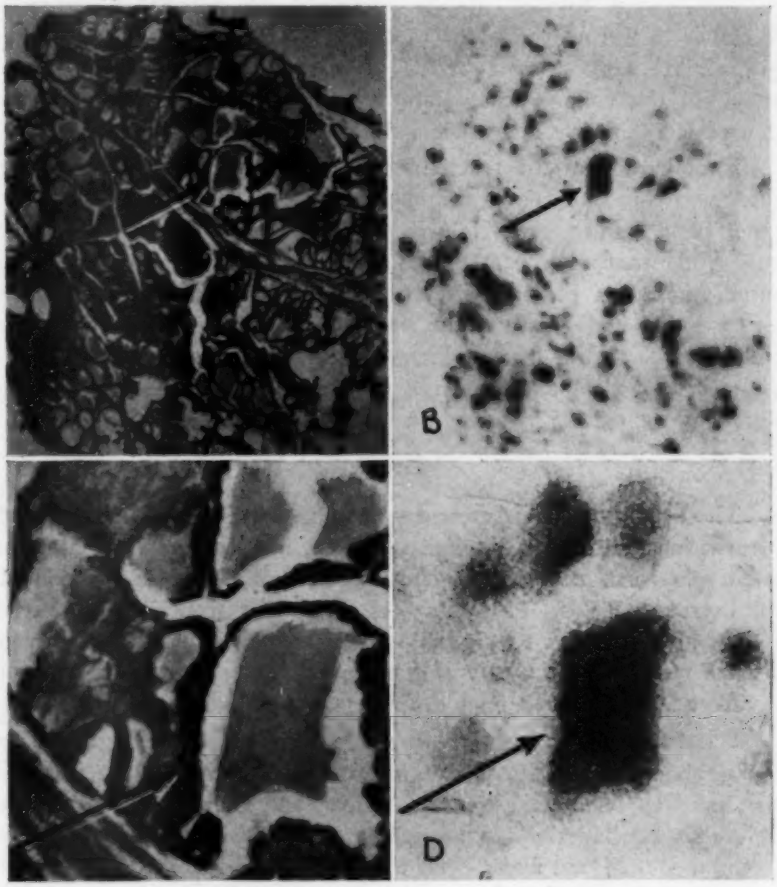

Fig. 2.—Sections of the thyroid gland of a patient (R. D.) with exophthalmic goiter and a basal metabolic rate of plus 40 per cent, and the corresponding radioiodine autographs. A comparison of the photomicrographs (*A* and *C*), which are typical of toxic diffuse hyperplastic goiter, and the radioautographs of these sections (*B* and *D*) again emphasizes that the radioiodine is deposited in the colloid. This is especially clear when comparing the arrow-indicated areas.

here represent studies of the thyroid gland of a patient (E. S.) with nontoxic diffuse colloid goiter and a basal metabolic rate of minus 7 per cent (fig. 1) and of a patient (R. D.) with moderately active exophthalmic goiter and a basal meta-

bolic rate of plus 40 per cent (fig. 2). The protocols of these patients have been presented in another communication.[9]

HISTOLOGIC RESULTS

Analysis of the autographs revealed that only true thyroid tissue affected the photographic plate. As in the case of the thyroid glands of rats,[3] the blood vessels, the nerves and the connective tissue stroma of the human thyroid glands contained no detectable radioiodine. Whether in diffuse colloid or in hyperplastic goitrous tissue, the autographic reactions were revealed as spots corresponding in size and shape to the colloid accumulations. In no instance did the groups of cells contain any detectable iodine by this method (figs. 1 and 2).

Examination of the reactions produced by the colloid showed that these varied in intensity in neighboring follicles (figs. 1 and 2). On the other hand, in any given follicle the reaction was fairly homogeneous, suggesting that each part of the colloid of one follicle contained the same amount of radioactivity (figs. 1 and 2). There was, however, an exception in the case of the single large vacuoles which were sometimes found in the large follicles. Such vacuoles did not affect the photographic plate and appeared as white spots in the dark area produced by the colloid present in a follicle (fig. 1, *G* and *H*).

COMMENT

These autographs demonstrate that most of the radioactive iodine present in diffuse colloid goiter as well as in exophthalmic goiter about twenty hours after its administration was deposited in the colloid of the thyroid follicles. If the cells of the follicles contained some radioiodine, also insoluble to the histologic reagents, it was apparently only a small proportion of that within the gland, since there was not enough to affect the zone of the photographic plate facing the cell accumulations.

Whatever iodine was in soluble form in the thyroid gland should have been extracted during the preparation of histologic sections since the fixatives used were alcohol, xylene and paraffin. The inorganic iodine should have been removed, therefore, while the iodine of thyroglobulin should not have been affected. In both the diffuse colloid goiter and the exophthalmic goiter less than 10 per cent of the radioactive iodine was in the inorganic form.[8] The rest was incorporated in diiodotyrosine or thyroxin.[8] It may be estimated that about 90 per cent of the radioiodine revealed by the autographic method was present as diiodotyrosine. Thus, a comparison of the chemical [8] and histologic findings indicates that most of the radioiodine passed through the epi-

9. Puppel, I. D.; Leblond, C. P.; Riley, E., and Curtis, G. M.: The Clinical Significance of the Functional Behavior of Adenomas of the Thyroid Gland, J. Lab. & Clin. Med., to be published.

thelium of the thyroid follicle to be deposited as diiodotyrosine in the colloid.

The question arises why somewhat different results have been obtained in these autographs of human thyroid glands and those of Hamilton and his group.[7] In our investigation the radioiodine, regardless of the type of goitrous tissue, was always well defined in areas of colloid. In Hamilton's material,[7a,c] on the other hand, where localization of radioiodine often appeared less sharply circumscribed in the follicles it nevertheless showed a preferential distribution for certain tissues. Thus the amount of radioiodine was greater in tissue areas of greatest activity, a fact corroborated by our chemical findings,[8] which show a lesser fixation of radioiodine in thyroid adenomas than in the surrounding thyroid tissue, which is presumably more active.

These differences in autographic results cannot be explained at this time. Can they be due to differences in method, dosage, reagents, time factors or strength of radioactivity used? Further investigation is necessary.

Finally, it should be noted that the radioiodine is homogeneously distributed throughout the colloid in any given follicle, since many histologists believe that there may be several kinds of colloid or colloid-like material in a given follicle. Our result suggests, on the contrary, that there is probably no difference between the colloid in the center of a follicle and that at the periphery. Material entering the colloid is apparently homogeneously distributed.

SUMMARY

Radioiodine was administered orally to patients with diffuse colloid and exophthalmic goiter before subtotal thyroidectomy. Subsequent autographic study of the removed glands showed that the iodine was rapidly deposited in a stable form in the colloid part of the thyroid follicle.

HEPATIC AND RENAL NECROSIS IN ALLOXAN DIABETES OF RABBITS

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WITHIN the three years that have elapsed since Dunn, Sheehan and McLetchie [1] first reported selective necrosis of the islets of Langerhans in rabbits given injections of alloxan, the literature has been flooded with pertinent articles. As the published material has already been reviewed several times,[2] it would serve no useful purpose to summarize it again now. Suffice it to say that the administration of alloxan is an effective and convenient means of producing permanent diabetes in many animals, with the result that the problem of diabetes itself can be studied experimentally with greater facility.

The necessary amount of alloxan has been carefully investigated by several experimenters, and at the present time it appears to be the consensus that in the rabbit an effective diabetogenic dose is 200 mg. of a 4 or 5 per cent solution administered intravenously.[3] Smaller doses, even if injected repeatedly, may not be followed by permanent diabetes,[4] and larger doses may result in death of the animal in a few hours or in severe necrosis of the epithelium of the renal tubules.[5] In an attempt to produce intercapillary glomerulosclerosis in rabbits we used the accepted method as outlined only to find that although most of the animals survived the hypoglycemic stage, many of them died in the next few days in uremia. Necropsy disclosed extensive necrosis and sometimes calcification of the epithelium of the renal tubules, partial or complete necrosis of the islets of Langerhans including not only the beta

1. Dunn, J. S.; Sheehan, H. L., and McLetchie, N. G. B.: Lancet **1**:484, 1943.

2. (*a*) Duff, G. L., and Starr, H.: Proc. Soc. Exper. Biol. & Med. **57**:280, 1944. (*b*) Gomori, G., and Goldner, M. G.: ibid. **54**:287, 1943. (*c*) Bailey, O. T.; Bailey, C. C., and Hagan, W. L.: Am. J. M. Sc. **208**:450, 1944. (*d*) Joslin, E. P.: New England J. Med. **230**:425, 1944.

3. Gomori and Goldner.[2b] Bailey and co-workers.[2c]

4. Bailey, C. C.; Bailey, O. T., and Leech, R. S.: New England J. Med. **230**:533, 1944.

5. (*a*) Goldner, M. G., and Gomori, G.: Endocrinology **33**:297, 1943. (*b*) Brunschwig, A., and Allen, J. G.: Cancer Research **4**:45, 1944.

cells but the alpha cells, and massive necrosis of the peripheral portions of the hepatic lobules. We record these results because the extensive renal changes occurred in many of our animals with a dose of alloxan that should be relatively harmless to the kidneys and because a thorough search of the literature discloses only one report of focal hepatic necrosis observed in rats treated with alloxan [2b] (in 2 of 49 given injections) and no reports of massive necrosis observed in the many animals used in such experiments.

MATERIAL AND METHOD

Each of 30 rabbits weighing between 2,150 and 3,560 Gm. received a single intravenous injection of a 4 per cent solution of alloxan in distilled water in an amount equivalent to 200 mg. per kilogram of body weight. The animal was allowed plenty of water and food in the form of pellets and greens for twenty-four hours before and after the injection. The blood levels of nonprotein nitrogen and glucose were determined before the experiment was started, and the latter was determined at frequent intervals during the first twelve hours after the injection. Subsequently it was determined daily during the first few days and then at weekly intervals. The blood nonprotein nitrogen level was first redetermined on the fourth day after several rabbits died in spite of relatively normal blood sugar levels. Thereafter the nonprotein nitrogen level was determined at sporadic intervals and at the time of death in all instances. An analysis of urine collected from the bladder was made to the time of necropsy. Hypoglycemic convulsions were treated with 3 to 5 cc. of 50 per cent dextrose, administered intravenously, and subsequent hyperglycemia was controlled first with insulin injection U. S. P. and after the first week with protamine zinc insulin. Two rabbits not treated with alloxan each received 10 cc. of a 50 per cent solution of dextrose intravenously on three successive days. Tissues were fixed in Helley's fluid and solution of formaldehyde U.S.P. diluted 1 : 10, and were stained with hematoxylin and eosin, Bensley's stain for alpha and beta granules in the islets of Langerhans, nile blue sulfate stain for fat in the liver and the kidneys and Best's carmine stain for glycogen in the liver and the kidneys.

CLINICAL OBSERVATIONS

The normal nonfasting blood sugar levels ranged from 105 to 151 mg. per hundred cubic centimeters and the nonprotein nitrogen levels from 41 to 58 mg. per hundred cubic centimeters. After the administration of alloxan there was initial hyperglycemia, the blood sugar rising as high as 500 mg. per hundred cubic centimeters which was followed by hypoglycemia with a blood sugar level of 30 mg. per hundred cubic centimeters or less, and subsequently by permanent hyperglycemia, with the level of blood sugar as high as 800 mg. per hundred cubic centimeters. In 10 rabbits hypoglycemic convulsions did not develop, whereas 20 rabbits were convulsed from one to five times. The convulsions first occurred as early as three hours and as late as sixteen hours after the injection of alloxan. Nine rabbits died of the initial hypoglycemia, while 21 survived, and in each of these diabetes developed. Sixteen of the 21 survivors died at sporadic intervals of from three days to thirty days after the injection, and 5 are still living.

Most of the animals that died within the first week refused to eat or drink and became drowsy and comatose before death. Although their blood sugar levels ranged from 230 to 500 mg. per hundred cubic centimeters, the urine of none of

the rabbits showed acetone or diacetic acid. The blood nonprotein nitrogen levels of the rabbits that died within the first two weeks of the experiment ranged from 130 to 342 mg. per hundred cubic centimeters, and those of the rabbits that succumbed after that time ranged from 100 to 160 mg. per hundred cubic centimeters. Most of the latter died in hypoglycemia from overdoses of insulin. Three of the rabbits with a greatly elevated blood level of nonprotein nitrogen had no urine in their bladders at the time of necropsy, and another had only about 4 drops present. In each of these instances the cages were dry. Analysis of the few drops of urine from the rabbit just referred to, and of urine from other rabbits, disclosed a specific gravity as high as 1.036, an acid or a neutral reaction, a cloud to a heavy precipitate of albumin, sugar as much as 1 per cent in those treated for hypoglycemia and microscopically leukocytes, erythrocytes, cylindroids and hyaline, granular or cellular casts. None of the urines contained acetone or diacetic acid.

PATHOLOGIC OBSERVATIONS

Macroscopic Changes.—There were no noteworthy gross changes in any of the organs. In some instances the kidneys were entirely normal, in others they were congested, and in others still they were considerably paler than normal. The capsules were not adherent, and in some cases the cut surfaces of the kidneys showed only slight radial streaking. The liver revealed no appreciable change in color, although in some cases it was distinctly softer than normal. None of the animals was jaundiced. The pancreas was sometimes not as pink as it is normally, often considerably softer and sometimes less bulky, so that occasionally one could not be certain that one was not handling a piece of fat instead of the pancreas. Cataracts were not seen in any of the animals, and the remaining organs showed no contributory changes.

Microscopic Changes.—(*a*) Pancreas: The islets of Langerhans showed changes progressing from degeneration to complete necrosis and disappearance. At five hours they were swollen and appeared more conspicuous than those in a normal organ. The cytoplasm was homogeneous, stained deep pink and tended to coalesce, thus obliterating the cell boundaries. Most of the nuclei were pyknotic, although a few had undergone rhexis. Generally the process was diffuse and involved both the alpha and the beta cells. Unscathed cells when present, however, were found at the periphery. The changes increased in severity so that within twelve hours there were notable shrinkage in size, fading or complete disappearance of the nuclei, and granularity of the cytoplasm. Within twenty-four hours most of the islets were completely necrotic (fig. 1 *A*) and further reduced in size, although there were generally some in which a few peripheral cells remained intact. From the third to the ninth day there were various mixtures of degeneration, necrosis and disappearance of the islets with, however, always a few present that were less damaged. Numerous sections disclosed an unequivocal paucity of islets and what appeared to be gradual replacement of the disappearing elements with pancreatic cells of an exocrine variety (fig. 1 *B*). These cells were seen to be intimately connected with the surrounding acinous cells. The younger ones were somewhat smaller, and their cytoplasm was more homogeneous, but as they aged, they became fewer and fewer and more and more of them disclosed granularity of cytoplasm until ultimately they became indistinguishable from the surrounding acinous cells. By the fourteenth day there were many instances in which no vestige of any islet could be found in the pancreas. By the twenty-first day, however, sections of two organs did show a fair number of small but relatively normal islets. It could not be ascertained whether these were regenerated or originally

undamaged structures. In no instance did the pancreas show hyalinization, leukocytic infiltration, mitosis or damage of the exocrine portion of the gland.

(*b*) Kidneys: Within five hours after the injection of alloxan the epithelium of all the proximal convoluted tubules of the affected kidneys was swollen to such a degree that the lumens were completely obliterated. The cytoplasm was granular, and the nuclei were already poorly stained, rhectic or entirely absent. There was granular material within the lumens of the more distal portions of the nephrons,

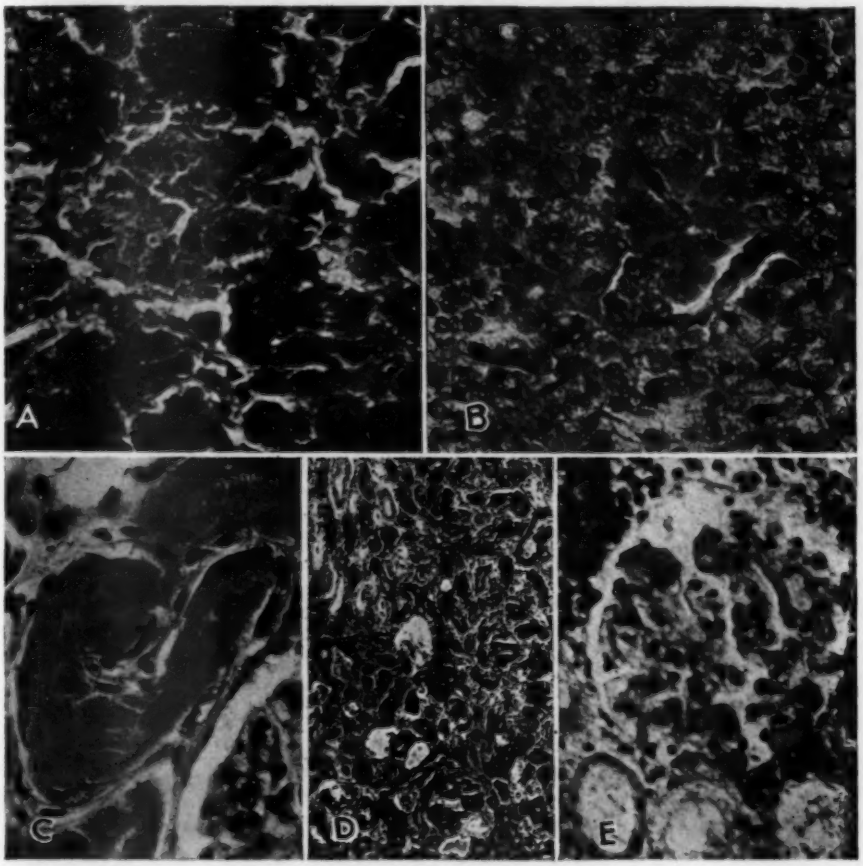

Fig. 1.—*A*, section of pancreas from a rabbit that died fourteen hours after an injection of alloxan, showing complete necrosis of an islet. Hematoxylin and eosin; × 400.

B, section of pancreas showing complete replacement of a necrotic islet with newly proliferated cells of an exocrine variety. Their nuclei are similar to those of the surrounding cells, but their cytoplasm is more homogeneous and less granular. Hematoxylin and eosin; × 400.

C, section of kidney showing necrosis of a proximal convoluted tubule and regeneration of the epithelium at the periphery. Hematoxylin and eosin; × 400.

D, section of kidney showing calcification of the necrotic epithelium of the proximal convoluted tubules. Hematoxylin and eosin; × 75.

E, section of kidney showing necrosis of a glomerulus and of the tubules and densely infiltrating polymorphonuclear leukocytes. Hematoxylin and eosin; × 400.

but the remaining structures were normal. Within fourteen hours the process was more advanced, the tubular epithelium in focal areas being completely necrotic and lying free in the lumen. At approximately the same time some of the epithelial cells became irregularly vacuolated, and the tubular lumens contained granular, hyaline and erythrocytic casts. At nineteen hours there was severe diffuse necrosis of practically all of the proximal convoluted tubules, and at thirty hours calcification of some of the necrotic tubules was already present. At three days in some cases the kidneys disclosed edema of the interstitial connective tissue, scattered collections of leukocytes and elongated, flattened cells at the periphery of the necrotic material within the tubules. These regenerating cells were more conspicuous by the fourth day (fig. 1 *C*). At this time vacuoles were also present in the less disturbed cells, and sections stained with nile blue sulfate revealed numerous blue to purple granules within both the tubular epithelium and the glomeruli. By the seventh day many of the tubules showed degeneration and necrosis, but the most striking change was the extensive calcification of the proximal convoluted tubules (fig. 1 *D*). Foci of leukocytes were observed throughout the cortex. Beyond the seventh day the changes were less spectacular, the kidneys being relatively normal in most cases. Hyaline casts were still present in the distal convoluted and collecting tubules. Here and there were a few foci of necrotic condensed tubules, surrounded by a few lymphocytes and plasma cells. In some sections there were only a few calcified tubules, with remaining elements relatively normal. The nile blue sulfate stain revealed no fat, and Best's carmine stain showed a few droplets of glycogen in some of the arched tubules of the rabbits whose diabetes was not completely controlled.

One rabbit that died at eleven hours deserves special mention, for sections of the kidneys showed almost complete necrosis of not only the tubules but the glomeruli as well. The tubular epithelium was granular and its inner border fuzzy. The nuclei were poorly stained or entirely absent, and the tubular lumens contained debris and erythrocytes. Similar cytoplasmic and nuclear changes were present in the glomeruli. There was extensive interstitial edema, and there were focal collections of neutrophils around many glomeruli and tubules (fig. 1 *E*).

Not all rabbits showed the changes described in the foregoing paragraphs. Among the 25 animals that died there were 14, or 56 per cent, that disclosed tubular necrosis, and in 8 of these 14, or 32 per cent of the total number of 25, the necrosis was diffuse and in 6, or 24 per cent of the total, it was focal.

(*c*) Liver: Within five hours after the injection of alloxan sections of the liver disclosed diffuse coagulative degeneration to necrosis of the peripheral two thirds of the hepatic lobules (fig. 2 *A*). The demarcations between the healthy and the affected zones were not sharp. In the latter the cells were greatly swollen; their boundaries were indistinct; their cytoplasm was finely granular and pink; their nuclei were shrunken and pyknotic, with lighter staining or with staining entirely absent, and prominent capillaries contained an excess of neutrophils. At ten hours the changes were essentially the same except that the cytoplasm of the cells nearer the portal radicles first became vacuolated and then completely disintegrated, so that there were definite areas of complete necrosis. At the junction of the normal and the necrotic tissue there was a conspicuous zone of polymorphonuclear leukocytes (fig. 2 *B*). Similar but somewhat more extensive changes were encountered in the livers of the animals dying within the first four days. In 2 rabbits that died on the fourth day there was not only necrosis of the peripheral portions of the lobules but marked deposition of fat within the centralmost portions (fig. 2 *C*). In a few of the animals that died after the seventh day there was

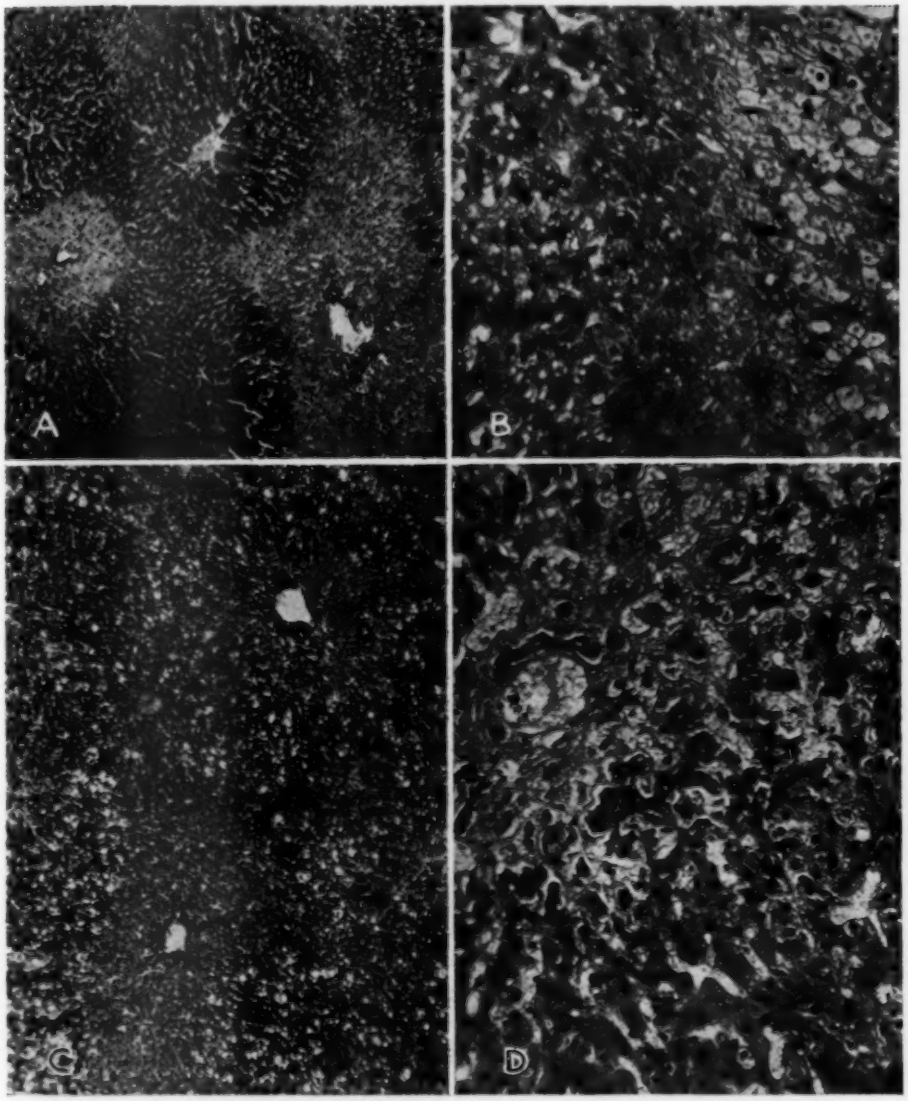

Fig. 2.—*A*, section of liver showing coagulative necrosis of the peripheral portions of the hepatic lobules. Hematoxylin and eosin; × 50.

B, section of liver showing liquefactive necrosis, leukocytic infiltration and early fatty metamorphosis of otherwise relatively normal central liver cells. Hematoxylin and eosin; × 200.

C, section of liver showing periportal necrosis and marked fatty metamorphosis of the central portions of the lobules. Hematoxylin and eosin; × 50.

D, section of liver showing proliferating hepatic cells invading recent, loose, connective tissue in a portal radicle. Hematoxylin and eosin; × 200.

similar central deposition of fat without, however, peripheral necrosis of the lobules. With nile blue sulfate, these deposits stained blue to purple. The livers of most of the rabbits that died after the seventh day had an essentially normal histologic appearance, with a return of the normal glycogen content, which during the stage of necrosis was completely or almost completely depleted.

Sections of the liver of 1 animal that died sixteen days after the administration of alloxan showed rather prominent portal radicles (fig. 2 *D*). These contained the normal portal triads, which were surrounded, however, with an abundant amount of recent, loose connective tissue. The liver cells adjacent to these areas were smaller than normal; their cytoplasm was deep pink, and their nuclei were relatively large. They were irregularly arranged, and although directly connected with the adjacent liver cell cords, they did not form a continuation of the regular lobular pattern. They appeared to be in the process of proliferation and to be invading the portal radicles, where they often produced new bile ducts.

As in the kidneys, the changes mentioned were not present in the livers of all the animals that died. When encountered, however, the process was diffuse; that is, it involved all portions of the liver with equal severity. Of the 25 animals that died, hepatic necrosis was found in 10, or 40 per cent, and was present in all but 1 of the animals that died within the first four days. Fatty metamorphosis was encountered in 6 rabbits, or 24 per cent, and was found as late as the thirtieth day after injection.

Sections of other organs, including the adrenal glands, showed no pertinent changes.

COMMENT

The response of the rabbits to alloxan—temporary hyperglycemia and hypoglycemia followed by permanent hyperglycemia—was no different from that in other experiments already published. The explanations for this typical curve have also been adequately covered [6] and will not be repeated here. The initial hyperglycemic stage is of little consequence, for it does no harm, but the hypoglycemic stage is serious; although it is only temporary, it may last from a few to twenty-four hours and in spite of the administration of dextrose may prove fatal to the animals. Some authors have intimated that a single intravenous injection of dextrose solution will tide the animals over this period.[2c] We used as many as a dozen injections, and still the animals became convulsed, indicating that constant supervision is necessary for at least thirty hours after an injection of alloxan. Signs of impending hypoglycemic convulsions were noted in not a few of the rabbits. The animals appeared rather apprehensive, nervous and overly alert. Their posture was usually that of sitting with the front legs stretched to a maximum and the neck arched backward. Their heads moved backward and forward in short, jerky, purposeless movements, and they ground their teeth vigorously. When dextrose was not administered, generalized convulsions usually ensued in from fifteen to sixty minutes.

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6. Hughes, H.; Ware, L. L., and Young, F. G.: Lancet **1**:148, 1944. Goldner, M. G., and Gomori, G.: Endocrinology **35**:241, 1944. Hard, W. L., and Carr, C. J.: Proc. Soc. Exper. Biol. & Med. **55**:214, 1944.

Diabetes developed in all of our rabbits that survived the initial hypoglycemia. For its control we used protamine zinc insulin, administered once daily. Unlike man, rabbits are most difficult to stabilize, probably because of the irregular intake of food. We found it not too infrequent that animals receiving 1 unit of insulin regularly would have high blood sugar levels, 400 to 500 mg. per hundred cubic centimeters, for several days or even weeks and then with continuation of the same regimen hypoglycemic convulsions would suddenly develop and many of the rabbits would die. It would be ideal to determine blood sugar daily and administer the insulin accordingly. This, however, we found impracticable, for it was not long before the veins of the ears were so destroyed that to get enough blood for even a weekly microdetermination of sugar was often difficult.

The changes in the islets of Langerhans have been described minutely several times.[7] Most authors have noted that the central portions of the islets are attacked first, the peripheral cells being left intact, and that only the beta cells are disturbed, while the alpha cells remain unscathed. Although this was true also in those of our animals that died within a few hours after the injection of alloxan, in those whose death was delayed there was complete necrosis of all the islet cells. Mitosis or hyalinization of the islets was not observed in any case. There was, however, complete disappearance of the necrotic material, so that in a few cases not a single islet of Langerhans could be found. This process was gradual, and it appeared to consist not merely of absorption of the debris and collapse of the "unsupported" sides but of actual proliferation of the adjacent acinous cells. All stages could be traced from the appearance of small dense cells in direct continuation with the pancreatic acini to their gradual enlargement and maturation until they became indistinguishable from the regular exocrine cells. Simultaneously they gradually encroached on the remains of the islets until the detritus was completely replaced with the newly formed cells. Although the islets of Langerhans were more numerous in the animals that died after the eighteenth day than they were in those that died sooner, it could not be ascertained whether there was proliferation of the islet cells or whether in these animals all the islets were not initially destroyed.

The changes in the kidneys bore a striking resemblance to those observed in mercury bichloride poisoning. There is no question that uremia induced by the extensive tubular necrosis was the cause of death in at least 8 animals. This side effect, therefore, cannot be overemphasized. That in many of the surviving animals probably similar but perhaps not quite as extensive renal changes developed with recovery, is evidenced

7. Dunn, J. S.; Kirkpatrick, J.; McLetchie, N. G. B., and Telfer, S. V.: J. Path. & Bact. **55**:245, 1943. Gomori and Goldner.[2b] Bailey and co-workers.[2c]

by the obvious regeneration of the tubular epithelium occurring at a time when the necrosis was still apparent and by the calcification still present in the proximal convoluted tubules of the kidneys of 1 rabbit that died thirty days after the injection of alloxan. Except for these calcific deposits the rest of the renal structure was entirely normal, indicating that regeneration was complete.

The alterations in the liver were diffuse and appeared to consist of four distinct processes. First, there was general depletion of the glycogen content of the liver. Second, there was initially coagulative and then liquefactive necrosis of the peripheral portions of the hepatic lobules with often a zone of inflammation along the margin of the dead tissue. This perilobular involvement was similar to that seen in women in eclampsia and in animals following intravenous injection of chloroform.[8] Third, there was severe fatty metamorphosis of the central portions of the lobules, which first became manifest while the necrosis was still apparent. Whereas fatty changes in the liver have been noted by several observers,[9] massive hepatic necrosis has not been previously reported. Fourth, there was some evidence of healing in 1 rabbit that died in hypoglycemia sixteen days after the injection of alloxan. The portal radicles were prominent and filled with loose, recent fibrillary connective tissue. Encroaching on and gradually infiltrating this were evident young, regenerating liver cells and newly formed bile ducts, resulting in the formation of early portal cirrhosis.

Finally, while extensive necrosis was observed in the pancreas, the kidneys and the liver, there was no regularity of involvement of these organs. None was necessarily affected to the exclusion of the others, nor was each equally involved in all animals. In all three organs the necrosis was probably caused by alloxan or a derivative of alloxan acting directly on tissue cells. The specific manner in which the agent affects the islets of Langerhans is most obscure. It might act on the liver cells during the process of detoxification, if the liver acts in this capacity, and it might act on the tubular epithelium of the kidneys while being excreted. As far as we know, however, there is no adequate proof for either hypothesis.

SUMMARY

Thirty rabbits received a single intravenous injection of 200 mg. of alloxan per kilogram of body weight in a 4 per cent solution. Of 25 animals that died within thirty days, 14 per cent disclosed necrosis of the epithelium of the proximal convoluted tubules of the kidneys similar

8. Karsner, H. T.: Human Pathology, ed. 5, Philadelphia, J. B. Lippincott Company, 1938, p. 686.

9. Duff and Starr.[2a] Bailey and co-workers.[2c] Goldner and Gomori.[5a]

to that seen in mercury bichloride poisoning. In at least 8 of these, the necrosis was severe enough to account for the uremia in which the animals died.

Ten of 11 animals that died within the first four days after the injection of alloxan disclosed extensive necrosis of the peripheral portions of the hepatic lobules. Such changes have not been previously reported observed in alloxan diabetes.

In the pancreas the disappearance of necrotic islets of Langerhans may be accounted for by the traceable proliferation of adjacent exocrine cells rather than by mere absorption of the detritus and collapse of the "unsupported" walls.

HEART WEIGHT

II. The Effect of Tuberculosis on Heart Weight

PEARL M. ZEEK, M.D.
CINCINNATI

ONE of the beliefs shared by many pathologists and clinicians is that tuberculous patients are liable to have small hearts. A survey of the literature on this subject discloses four hypotheses concerning the nature of the so-called small hearts found in tuberculous patients: (1) that these are congenitally small hearts and that the resultant poor circulation in the lungs and elsewhere has predisposed to the development of tuberculosis; (2) that these hearts are really not small at all but just look that way by roentgenogram because of a change in position which produces the so-called dropped heart; (3) that small hearts in bedridden tuberculous patients may represent muscular atrophy of relative disuse; (4) that these hearts have decreased in weight because of the emaciation associated with the disease, which has similarly affected other organs and tissues, including other muscular structures.

The first hypothesis is an old one. Two of its adherents were Guarini [1] and Brehmer,[2] the latter believing that the heart of the tuberculous patient is too small for the lungs, so that there occurs a relative anemia of the lungs which predisposes to tuberculosis. Sweeney [3] challenged this opinion on the grounds that the more vascular tissues are the ones that are susceptible to tuberculosis, not the relatively avascular ones.

The second hypothesis is based on roentgenologic studies, which do not yet provide accurate criteria of heart weight.

The third hypothesis was suggested by White,[4] who said, however, that such cardiac atrophy is infrequent and in most cases slight.

The most commonly expressed hypothesis is the fourth one, but little statistical or experimental evidence has been reported to support it. In 1854 Peacock [5] studied the weights of healthy and diseased hearts and concluded that "the weight of the heart in phthisis was intermediate between that of cases dying of acute and other chronic disease,"

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1. Guarini, cited by Potthoff.[14]

2. Brehmer, H.: Separat-Abdruck aus den Mittheilungen aus Dr. Brehmers Heilanstalt für Lungenkranke, Weisbaden, J. F. Bergmann, 1889.

3. Sweeney, J. A.: Am. Heart J. **20**:345, 1940.

4. White, P. D.: Heart Disease, New York, The Macmillan Company, 1937.

5. Peacock, T. B.: Monthly J. M. Sc. **19**:193, 313 and 403, 1854.

and he pointed out that pulmonary obstruction of blood flow tends to cause cardiac enlargement but is counteracted by emaciation. It making up his series of cases with "healthy heart" he did not exclude cases of hypertension and arteriolar sclerosis; therefore the heart weights in the cases of tuberculosis in his series tended to be somewhat greater than those of the present study. In 1892 Potain [6] noted that the hearts of phthisic patients were unusually small and attributed this to the cachexia which accompanies the disease. He expressed the belief that if the progress of the disease was very slow, large hearts might be found, with the enlargement being due to extrapulmonary causes.

Pearl and Bacon,[7] in a statistical study of the records of 5,000 routine autopsies, found 1,341 in which tuberculous lesions were noted. They studied the relation of the heart weight to the weights of other viscera in tuberculous and nontuberculous persons but realized that in both groups there probably were nontuberculous lesions of the cardiovascular system which were of far greater significance in relation to the variations in heart weight than was tuberculosis. Nevertheless, they concluded that active tuberculosis tended definitely to be associated with a reduced absolute weight of the heart, compared with the heart weights of patients who had inactive tuberculous lesions. The authors stated that their study did not show whether persons with tuberculosis had small hearts because they had tuberculosis, or whether they had tuberculosis because congenitally they had relatively small hearts. In a subsequent study of these same records they found variations in the mean heart weights of different age groups, but they did not rule out hypertension and arteriolar sclerosis, and, consequently, greater than normal mean heart weights occurred in age groups in which these conditions might be expected to produce an effect on the weight of the heart. Body weights were not known, and no mention was made of body nourishment or of body length. However, it seems evident that in their series of cases active tuberculosis was associated with decreased heart weight.

Krieger [8] studied the atrophy of human viscera associated with inanition. In 39 cases of tuberculosis there was an average decrease in heart weight of 31.9 per cent, while the average decrease in body weight was 43 per cent. He used Gärtner's formula (based on height) for normal body weight and heart weight.

Tallquist,[9] in a study of the small heart, listed tuberculosis as one of the associated pathologic conditions. Fishberg [10] stated that usually in

6. Potain, P. C., cited by Porter and Gordon.[15]
7. Pearl R., and Bacon, A. L.: Johns Hopkins Hosp. Rep. **21**:157 and 297, 1920.
8. Krieger, M.: Ztschr. f. ang. Anat. u. Konstitutionslehre **7**:87, 1920.
9. Tallquist, T. W., abstracted, J. A. M. A. **7**:1380, 1921.
10. Fishberg, M.: Pulmonary Tuberculosis, Philadelphia, Lea & Febiger, 1922.

tuberculosis "the heart is small, weak and atrophic, the individual muscle fibers being atrophied and fatty." Simon and Baum [11] studied the electrocardiograms of 250 persons with chronic pulmonary tuberculosis but found few departures from normal, and these were not striking. Anderson,[12] who also studied electrocardiograms, stated that "tuberculosis has no specific effect on the heart aside from that produced by any chronic, debilitating malady."

Hawes [13] stated that "in most instances the small heart in tuberculosis is due to the wasting effect on heart muscle, just as the leg, arm, and shoulder muscles waste away from the same cause." Potthoff [14] studied the heart shadows in the roentgenograms of 600 tuberculous men. He compared the mean transverse diameters of the heart shadows in the various body length groups with Hammer's figures for the same groups of healthy males and found that in each group the mean for tuberculous men was smaller than the corresponding mean in Hammer's series. Porter and Gordon [15] studied the teleroentgenograms of 400 tuberculous patients but drew no conclusions as to the effect of tuberculosis on the size of the heart. They did not rule out hypertension and other causes of myocardial hypertrophy, and they made no mention of body nourishment except as it was indicated in body weight. Higgins [16] studied the relative weights of the right and left ventricles in a series of 600 autopsies of tuberculous patients and stated that the ratio of the weight of the right to that of the left ventricle is more significant than the total cardiac weight when one is looking for evidence of right ventricular hypertrophy in emaciated bodies.

PRESENT INVESTIGATION

In a series of 926 adults with relatively normal hearts (the cases were collected from the records of the Cincinnati General Hospital for a previous study on the weight of the normal human heart [17]) there were 221 in whom active tuberculous lesions were presented at autopsy. These lesions varied from active foci in lymph nodes, found only microscopically, to far advanced lesions which were the cause of death. No adult was included in this series of 221 in whom only inactive lesions were found.

In the preceding study of the normal heart three factors found to produce effects on heart weight were sex, body length and body nourish-

11. Simon, S., and Baum, F.: Am. Rev. Tuberc. **17**:159, 1928.
12. Anderson, A. R.: Am. Rev. Tuberc. **20**:728, 1929.
13. Hawes, J. B., Jr: New England J. Med. **207**:874, 1932.
14. Potthoff, F.: Beitr. z. Klin. d. Tuberk. **88**:187, 1936.
15. Porter, R. E., and Gordon, W. H: Am. Rev. Tuberc. **36**:82, 1937.
16. Higgins, G. K: Am. Rev. Tuberc. **49**:255, 1944.
17. Zeek, P. M.: Arch. Path. **34**:820, 1942.

ment. Table 1 presents the group distribution of the cadavers with active tuberculous lesions and the nontuberculous cadavers of the present series according to these three factors and also gives the mean heart weight for each group. The bodies classified in the preceding study as "obese" and "muscular" respectively and also those whose lengths were at the extremes of the body length distribution curves were not included in table 1 because there were too few in each category

TABLE 1.—*Heart Weight in Tuberculous and Nontuberculous Bodies*

	Males				Females			
	Emaciated		Normally Nourished		Emaciated		Normally Nourished	
	Tuberculous	Nontuberculous	Tuberculous	Nontuberculous	Tuberculous	Nontuberculous	Tuberculous	Nontuberculous
Total number	73	63	55	297	60	55	23	199
Body length, 145-149 cm.								
Number of cases	..	..	..	..	3	3	1	14
Mean heart weight, Gm.	.....	.....	.....	.....	208.3	230.0	210.0	248.9
Body length, 150-154 cm.								
Number of cases	3	3	1	5	6	10	3	40
Mean heart weight, Gm.	228.3	258.3	285.0	299.0	198.3	209.0	226.7	246.3
Body length, 155-159 cm.								
Number of cases	4	5	..	11	16	12	6	47
Mean heart weight, Gm.	240.0	230.0	.....	298.2	212.5	225.4	256.7	259.7
Probable error of means, Gm.	...	...	...	...	6.3	4.6	8.6	3.9
Body length, 160-164 cm.								
Number of cases	14	9	11	40	16	16	5	56
Mean heart weight, Gm.	239.6	252.8	300.5	312.4	209.4	213.1	272.0	266.5
Probable error of means, Gm.	6.0	6.5	8.4	6.3	4.5	6.9	12.7	3.7
Body length, 165-169 cm.								
Number of cases	18	19	18	66	12	11	5	24
Mean heart weight, Gm.	252.2	265.0	296.9	304.8	218.3	230.0	274.0	274.6
Probable error of means, Gm.	6.5	6.0	5.1	3.7	6.0	4.4	7.5	5.3
Body length, 170-174 cm.								
Number of cases	18	20	17	82	5	..	3	10
Mean heart weight, Gm.	263.9	258.5	307.1	322.4	245.0	.....	246.7	288.0
Probable error of means, Gm.	5.7	7.5	4.7	2.9				
Body length, 175-179 cm.								
Number of cases	4	3	4	58	2	3	..	8
Mean heart weight, Gm.	308.8	285.0	322.5	330.9	282.5	258.3	.....	300.6
Body length, 180-184 cm.								
Number of cases	8	3	2	20				
Mean heart weight, Gm.	278.1	240.0	302.5	344.5				
Body length, 185-189 cm.								
Number of cases	4	1	2	6				
Mean heart weight, Gm.	273.3	350.0	365.0	346.7				

to provide a valuable mean. Therefore the total number of bodies in table 1 is 825 instead of 926.

Within each body length group the difference in heart weight between emaciated tuberculous and emaciated nontuberculous bodies is less than that between emaciated tuberculous and normally nourished tuberculous bodies. To test the significance of these differences, the following formula was used:

$$\frac{\text{Mean}_A - \text{Mean}_B}{\sqrt{(\text{P.E.}_A)^2 + (\text{P.E.}_B)^2}}$$

This represents the difference between two mean heart weights divided by the probable error of that difference. Pearl says: "We may regard a difference of less than three times its probable error as probably not significant, one of between three and four times as probably significant, and one of more than four times as for practical purposes certainly significant."

Table 2 shows that the difference in mean heart weight between tuberculous and nontuberculous persons of similar states of body nourishment is not significant, while the difference between emaciated tuberculous and normally nourished tuberculous persons is definitely significant. Therefore, tuberculous persons can be expected to have small hearts only if they are emaciated. When they are normally nourished, their

TABLE 2.—*Significance of the Difference in Mean Heart Weight Between Tuberculous and Nontuberculous Groups*

	$\frac{\text{Mean}_A - \text{Mean}_B}{\sqrt{(\text{P.E.}_A)^2 + (\text{P.E.}_B)^2}}$ (Male)*			$\frac{\text{Mean}_A - \text{Mean}_B}{\sqrt{(\text{P.E.}_A)^2 + (\text{P.E.}_B)^2}}$ (Female)*		
	Body Length, Cm.			Body Length, Cm.		
	160-164	165-169	170-174	155-159	160-164	165-169
When A = emaciated nontuberculous cadavers, and B = emaciated tuberculous cadavers	1.2	1.5	0.6	1.7	0.5	1.7
When A = normally nourished nontuberculous cadavers, and B = normally nourished tuberculous cadavers	1.1	1.3	2.8	0.3	0.4	0.6
When A = normally nourished tuberculous cadavers, and B = emaciated tuberculous cadavers	5.9	5.4	5.8	4.2	4.6	5.8
When A = normally nourished nontuberculous cadavers, and B = emaciated nontuberculous cadavers	6.6	5.6	7.9	5.7	6.8	6.5

* See text for explanation of the formula.

heart weights can be expected to be within the normal range. Table 2 shows also that within each body length group there is a significant difference between the mean heart weight of normally nourished nontuberculous persons and that of emaciated nontuberculous persons. Therefore emaciation can be expected to be associated with decreased heart weight whether tuberculous or nontuberculous in origin.

Since the weights of the bodies in this series are not known, there is no criterion for comparing degrees of emaciation in tuberculous and nontuberculous persons. However, the departures from normal in heart weights can be compared within certain limits. Among the 926 subjects collected for these studies on heart weight there were 257 described as emaciated. Table 3 shows the distribution of these in relation to normality of heart weight. The weight of each heart was compared

with the mean heart weight for normally nourished bodies of the same sex and body length.[17] The weight was considered normal if it fell within the limits of the standard deviation (40 Gm. for males, and 30 Gm. for females).

In 70 per cent of the 257 cadavers showing emaciation from any cause, the heart weights were less than the lower limits of normal for bodies of similar sex and body length. Of those heart weights which were within the normal range, an additional 18 per cent (25 males and 22 females) were below the mean of their respective groups. Therefore, other factors remaining constant, hearts in emaciated bodies can be

TABLE 3.—*Heart Weight in Emaciated Bodies*

Heart Weight	Males		Females		Total	
	No.	%	No.	%	No.	%
Less than normal (mean − S. D.)	95	70	85	70	180	70
Normal (mean ± S. D.)	38	28	31	26	69	27
More than normal (mean + S. D.)	3	2	5	4	8	3
	136	100	121	100	257	100

TABLE 4.—*Amount of Decrease in Heart Weight in Various Diseases Associated with Emaciation*

Grams Below Normal Heart Weight Range	Cases of Active Tuberculosis	Cases of Cancer	Cases of Addison's Disease
1-9	10	1	..
10-19	13	7	..
20-29	16	5	1
30-39	20	6	2
40-49	11	4	..
50-59	11	7	1
60-69	4	2	..
70-79	4	6	1
80-89	3	1	..
90-99	2	1	..
100-109	3	1	..
Total	97	41	5

expected to weigh less than hearts in normally nourished ones. Among the 180 emaciated bodies with heart weights below the normal range, 97 presented active lesions of tuberculosis, 41 cancer, 2 both of these conditions and 5 Addison's disease. Table 4 presents the amount of decrease in heart weight associated with these conditions. When the mean decrease in heart weight in the emaciated tuberculous bodies was compared statistically with that in the emaciated bodies with cancer no significant difference was found. However, the numbers in these groups are small, and the standard deviations are large. Also the degree of emaciation, which was not evaluated in this series, probably plays a significant role in determining the amount of

decrease in heart weight, although it has been found by Gray and Mahan [18] and by others that the heart weight does not diminish to the same degree as does the body weight.

In table 4 it is interesting to note how great may be the decrease in heart weight associated with emaciation. There is now in progress in this laboratory a study of small hearts, collected from autopsy material over a period of years, to determine what microscopic and clinical manifestations may be associated with decrease in cardiac weight.

SUMMARY

When there is emaciation, tuberculous or nontuberculous in origin, and when no cause for myocardial hypertrophy is present, heart weight can be expected to be less than normal. The decrease in heart weight is significantly associated with emaciation but not with tuberculosis as such. Similar decrease in the heart weight is found when emaciation is associated with cancer. In a tuberculous person who shows a normal state of bodily nourishment no diminution in heart weight is to be expected.

18. Gray, H., and Mahan, E.: Am. J. Phys. Anthropol. **1**:271, 1943.

GENESIS OF AORTIC PERFORATION SECONDARY TO CARCINOMA OF THE ESOPHAGUS

Report of Observations in Two Cases

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A TERMINAL complication of carcinoma of the esophagus is rupture into one or more adjacent structures or regions. The lesion may perforate into the mediastinum, the trachea, the bronchus, the lung substance by extending directly through the pleura, or even into the aorta or other large blood vessel.

Our files contain records of 46 cases of carcinoma of the esophagus in which necropsy was done. Perforation had occurred in 22 of these, and in a few more than one structure was involved. Both the mediastinum and the lung were perforated in 1 case, the trachea and the mediastinum in 2, and in 1 instance the tumor had eroded into the aorta as well as into the trachea. The relative frequency of involvement of the structures named is as follows:

Mediastinum	9
Trachea or bronchus	10
Lung	5
Aorta	2

From this tabulation it is evident that erosion of the aorta is one of the rarer complications. It had occurred twice in the 46 cases. Carr and Hanford [1] have adequately summarized the statistical frequency of this form of complication by quoting the figures given by various authors.

The number of cases of perforation of the aorta due to carcinoma of the esophagus appearing in the literature is not very great. Knaut [2] up to 1896 had collected 34 such examples. Carr and Hanford [1] gathered 17 cases reported between 1896 and 1922 and added a case of their own.

From the Department of Pathology, Medical College of the State of South Carolina.

1. Carr, J. G., and Hanford, C. W.: Am. J. M. Sc. **164**:340, 1922.

2. Knaut, B.: Ueber die durch Speiseröhrenkrebs bedingten Perforationen der benachbarten Blutbahnen, nebst einer Beobachtung von primärer Oesophagusdilatation und von Leukoplakia oesophagi, Inaugural Dissertation, Berlin, Vogt, 1896.

Since then 6 additional cases,[3] (including 1 case reported by Barron, which was overlooked in the aforementioned survey) have appeared. The 2 cases to be reported in the following pages bring the total to 60 recorded cases. These 2 cases are of further interest because the postmortem observations illustrate the genesis of the perforation.

REPORT OF CASES

CASE 1.—C. W. J., a Negro woman 76 years of age, was admitted to Roper Hospital in March 1945 because of progressive dysphagia since October 1944. This was accompanied by recurrent attacks of sharp, stabbing substernal and precordial pain usually lasting ten to fifteen minutes and not related to ingestion of food. Occasional vomiting of food and blood-tinged material occurred, with much froth in the vomitus. For the last two to three months the stools had been very dark, almost tarry.

Examination on admission revealed marked deviation of the trachea to the right. The lungs were clear to percussion and auscultation. The heart was shifted slightly to the right of the midsternal line, and a systolic murmur was heard at the aortic and apical areas. The blood pressure was 165 systolic and 80 diastolic. The abdomen presented nothing unusual

Blood counts gave 4,060,000 and 3,800,000 erythrocytes and 7,800 leukocytes per cubic millimeter. Urinalysis showed no abnormality on several occasions. The Kline test was positive, whereas the Wassermann reaction was negative.

While the patient was in the hospital a cough developed, continuous and persistent, with expectoration of considerable amounts of mucoid to mucopurulent sputum, sometimes blood tinged and at other times containing yellowish particulate material. On the second day after admission she began to complain of a dull aching pain in the right axilla and under the sternum. On the tenth hospital day she had a sudden massive hemorrhage from the nose and the mouth and died immediately.

Necropsy (eighteen hours after death).—The body was that of an emaciated Negro woman.

On the anterior surface of the esophagus, just distal to the bifurcation of the trachea, was a circumscribed, annular ulcerating neoplasm measuring 6 cm. in the greatest diameter. The edges of the lesion were elevated, rounded, gray-white and crisp, and the base consisted of discolored, gray-brown degenerating tissue. The tumor had eroded into the right main bronchus, producing a bronchoesophageal fistula measuring 8 mm. in diameter. The esophagus was densely bound down by neoplastic tissue to the aorta in this region, and here was found a sinus tract between the lumen of the esophagus and that of the aorta. This communication measured 3 mm. in diameter, and on the intimal surface of the aorta surrounding the perforation was a heaped, reddish thrombotic nodule measuring 4 mm. in all diameters.

The opening of the bronchoesophageal fistula was situated 2 cm. distal to the carina. Both bronchi and all the bronchioles were filled with clotted blood.

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3. (*a*) Barron, M.: J. A. M. A. **67**:1585, 1916. (*b*) Meyer, J. J.: ibid. **79**: 1335, 1922 (*c*) Nonnis, G.: Gior. di clin. med. **9**:585, 1928. (*d*) Polson, C. J., and McIntosh, W.: Lancet **2**:960, 1931. (*e*) Fujishiro, Z.: Oto-rhino-laryng. **11**:151, 1938. (*f*) Schattenberg, H. J., and Ziskind, J.: Am. J. Clin. Path. **9**:615, 1939.

The blood had been aspirated even into the alveoli. In addition the lungs presented metastatic tumor nodules in the parenchyma. Tumor metastases were also found in the kidneys, in the left adrenal gland and in the periesophageal lymph nodes.

The stomach and the small intestine contained approximately 1,500 cc. of fluid and clotted blood.

Microscopically, the esophageal tumor was composed of columns of epithelial cells involving the entire thickness of the wall. Epithelial "pearls" were absent, and mitotic figures and tumor giant cells were numerous. Several acute abscesses were present in the wall of the esophagus in the region of the neoplasm.

Sections of the aorta at the edge of the perforation showed a thickened adventitia infiltrated by similar anaplastic tumor cells arranged in cords and sheets. Tumor cells were also found in perineural and perivascular lymphatics. Many thin-walled vessels, presumably the veins of the vasa vasorum, were distended and thrombosed by tumor cells. In addition a few arterioles, also in the adventitia, were thrombosed, the thrombus being composed of young fibroblasts filling the lumen. An intercostal artery, in its short course through the adventitia and near the perforation, showed tumor cell invasion of its outer coat (fig. 1 *A*) and organizing thrombosis of its lumen (fig. 1 *B*). The media of the aorta was not involved by the neoplasm, and at the edge of the perforation it was lined by acellular amorphous eosinophilic material which did not stain for fibrin or elastic tissue. A small thrombus, composed of laminated fibrin and polymorphonuclear leukocytes and laden with a mixture of bacteria, lined the intima at the edge of the perforation. Bacteria of the same type were found along the entire thickness of the rupture. The eroded edge of the bronchus was lined by tumor cells throughout its thickness.

Case 2.—A. S., a Negro woman 38 years of age, came to the outpatient clinic of Roper Hospital on Dec. 22, 1942, complaining of sharp pain under the left breast in addition to mild dysphagia for the past four months. There had been slight nausea and vomiting on occasions. No respiratory complaints were elicited.

The patient returned to the clinic two months later and in the interim had lost 10 pounds (4.5 Kg.). Dysphagia became progressively more severe so that even water was swallowed with difficulty. She apparently refused hospitalization and three weeks later was seen in a critical condition in the Roper Hospital emergency room, where she died within a few minutes. The terminal event was marked by loss of a moderate amount of bright red blood through the nose and the mouth.

Examinations were noncontributory, and a roentgenogram of the chest when the patient was first seen revealed normal lung and heart fields.

At the time of her first clinical visit the blood showed a hemoglobin content of 11 Gm. per hundred cubic centimeters and a leukocyte count of 6,950 per cubic millimeter. The urine revealed nothing unusual.

Necropsy (eleven hours after death).—The body was that of an emaciated Negro woman weighing 90 pounds (41 Kg.).

The esophagus in its middle third presented an indurated, ulcerated lesion girdling the circumference of the wall and causing stenosis. This lesion measured from 3 to 6 cm. along the longitudinal axis of that organ. On the anterior aspect of the esophagus there was a defect in the tumor mass, 1 by 1.5 cm., communicating with an irregular cavity 2 by 3 by 4 cm. lying in the tissue between the aorta and the esophagus and densely adherent to the former. The

cavity contained recent and altered blood and was lined by ragged gray tissue. The periesophageal as well as the other posterior mediastinal and bronchial lymph nodes contained metastatic tumor.

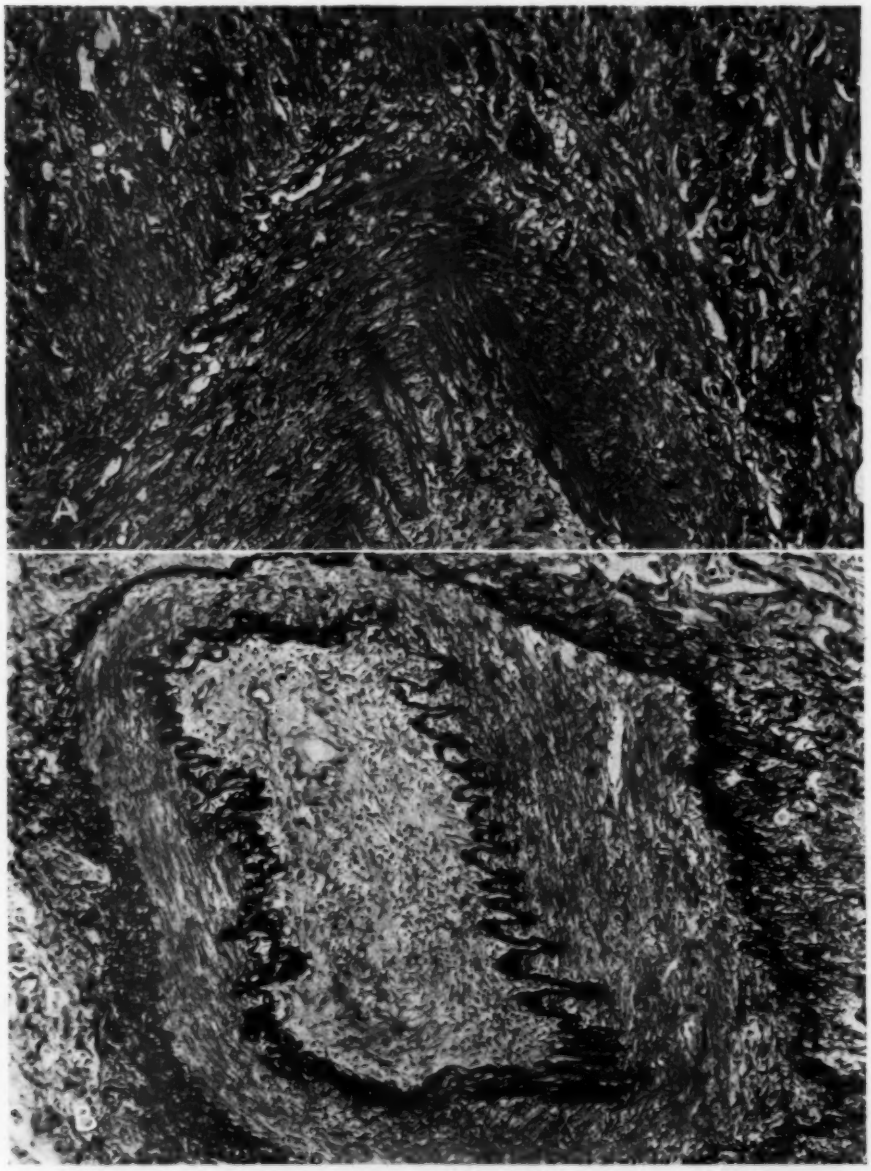

Fig. 1.—*A*, portion of the wall of an intercostal artery showing clumps of tumor cells in its outer layer on the right side of the photograph. Hematoxylin and eosin; × 100.

B, cross section of the intercostal artery, the lumen of which is occluded by an organizing thrombus. Weigert's elastic tissue stain; × 75.

The intima of the aorta at the junction of the arch and the descending thoracic portion showed an indefinite, irregular grayish discoloration 1 cm. in diameter. This was situated on the anteromesial aspect of the vessel, and in its center were two minute perforations of the intima, the larger 1 by 2 mm. in diameter. These erosions extended into the aforementioned mediastinal cavity which communicated with the esophagus.

The stomach and the small intestine contained about 2,000 cc. of fluid and clotted blood. The remaining viscera showed considerable pallor and there was no evidence of metastases.

Microscopically, the esophageal tumor consisted of masses of epithelial cells arranged in sheets and cords, infiltrating the entire thickness of the wall. Numerous mitoses and tumor giant cells in addition to an occasional "pearl" were noted.

A striking feature of the aorta at the point of rupture was the tremendous thickness of the adventitia, this coat being three times the width of the media. The thickening was due to an increased amount of dense collagenous connective tissue, muscle fibers and occasional elastic fibrils in addition to clumps and cords of tumor cells. Only the adventitia was invaded by the neoplasm. The vasa vasorum showed an apparent decrease in their number and most of those that were present showed their lumens occluded by organizing thrombus material, which consisted of proliferating fibroblasts. This was observed on serial sections of the aorta taken near the edge of the perforation. Another striking feature was the distention of venules and other thin-walled vessels in the adventitia caused by tumor thrombi (fig. 2). The media and the intima at the point of perforation showed an acellular amorphous necrotic debris, which again did not take the fibrin or the elastic tissue stains. The intima surrounding the erosion presented a thrombus composed of fibrin and polymorphonuclear leukocytes.

Gram-positive and gram-negative bacilli and cocci were sprinkled through the mural thrombus on the intima and in the necrotic debris along the edge of the perforation.

COMMENT

Carcinoma of the esophagus begins in the mucous membrane and may infiltrate the entire thickness of the esophageal wall. Spread of the tumor may then involve, by continuity of tissues, the surrounding structures, including the aorta. The only portion of the aorta that is invaded by tumor cells is the adventitia; the media and the intima are spared. This was true in both of our cases, as shown by serial sections of the aorta at the point of perforation. This was also true in those reported cases in which the aorta at the area of rupture was described microscopically.

Indeed a noteworthy finding in both of our cases was the thrombosed vasa vasorum, the thrombi in some of the vessels being due to tumor. In one instance the lumen of an intercostal artery was filled with proliferating fibroblasts. That intercostal arteries give rise to nutrient vessels to the aorta has been demonstrated by Robertson.[4]

4. Robertson, H. F.: Arch. Path. **8**:881, 1929.

The thrombosis of the vasa vasorum suggests the genesis of the aortic perforation. Vierhuff [5] in 1896 recognized that impairment of the blood supply of the aorta was an important factor leading to its rupture. He observed that only the adventitia of the aorta was invaded by tumor and that the media at the point of perforation was composed of necrotic material due to "defective tissue nourishment from destruction of the vasa vasorum."

According to Schattenberg and Ziskind,[3f] the perforation occurs usually as the tumor "disintegrates before the advancing ulcerative process" and in some cases because of suppuration of the tumor. The latter part of their statement obviously suggests perforation due to bacterial invasion of the tumor. This view was upheld by Barron,[3a] who demonstrated bacteria in the perforation and only a few tumor

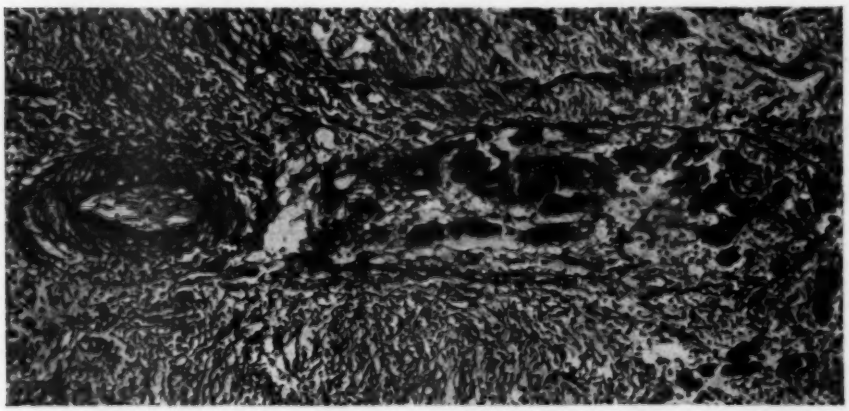

Fig. 2.—Section through the adventitia of the aorta in case 2. An arteriole occluded by proliferating fibroblasts is shown on the left. To the right is a thin-walled vessel distended with tumor cells; × 100.

cells in the adventitia while none were present in the media and the intima. He therefore concluded, since the carcinomatous invasion of the aorta was minimal and the amount of infection was profound, that the latter contributed greatly to the perforation.

It seems that of the authors mentioned only Vierhuff has considered the condition of the vasa vasorum in this disease process. Obviously, the aorta depends for its nourishment on the integrity of these vessels, and any disease that involves them will be ultimately reflected on that great vessel. Since there was a conspicuous decrease in the number of the vasa vasorum in the thickened adventitia and since most of those that were present were thrombosed either by

5. Vierhuff, W.: St. Petersburg med. Wchnschr. **13**:195, 1896.

proliferating fibroblasts or by invading neoplasm, we feel that the perforation in both cases was due to interference with the blood supply. Furthermore, we are of the opinion that examination of the aorta at the site of perforation will reveal thrombosed vasa vasorum in all cases.

Although bacteria were present in our sections of the aorta, they were most likely saprophytes or even postmortem invaders and not true pathogens. This is substantiated by the fact that amorphous material was present at the perforation which did not contain fibrin and further by the fact that the site showed no inflammatory cellular reaction.

CONCLUSIONS

Perforation of the aorta due to carcinoma of the esophagus is of infrequent occurrence. Study of 2 cases indicated that perforation of the aorta results from interference with the blood supply secondary to thrombosis of the vasa vasorum and not from tumor invading the entire vessel wall nor from bacterial infection.

Case Reports

ENTERITIS CAUSED BY SALMONELLA SUIPESTIFER WITH SECONDARY MONILIASIS

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IT IS the purpose of this paper to describe the results of a pathologic examination of a patient succumbing to Salmonella suipestifer infection complicated by moniliasis of the upper gastrointestinal tract. The latter was unsuspected during life.

Longcope [1] was the first to describe the clinical features of S. suipestifer infection. In 1902 he reported 2 cases. One patient came to autopsy; the other recovered. No ulcers were seen in the intestines, and only a few focal necroses were present in the liver. In 1918 Neukirch [2] reported a study of patients many of whom presented symptoms of typhoid. He cultivated an organism, which he called Bacillus erzindjahn, and which probably belonged to the parathyphoid group B. One of the patients, whose illness resembled most closely an attack of dysentery, showed at autopsy irregular fresh ulcers of the colon. Neukirch drew a parallel between S. suipestifer and B. erzindjahn as far as virulence and invasiveness was concerned. In 1937 Harvey [3] summarized the literature and added a report of new cases. The diseases caused by the Salmonella group were discussed by Bornstein [4] in 1943. Harvey noted the conspicuous absence of intestinal lesions in S. suipestifer infection and at the same time stated that "in only an occasional case" is the organism cultivated from the stool. The postmortem findings, according to Bornstein, are not characteristic. Seligmann, Saphra and Wassermann,[5] who analyzed 1,000 cases of Salmonella infection collected at the New York Salmonella Center, divided the clinical manifestations into (*a*) predominantly gastroenteric signs, (*b*) localized processes due to contact or hematogenous spread and (*c*) typhoid-like symptoms and symptoms of septicemia without localizing signs. For the sake of completeness, the asymptomatic carrier state was included. The case at hand combines gastroenteric symptoms with those of septicemia.

REPORT OF A CASE

E. F., a 60 year old white married woman, a dressmaker, was admitted to the medical service of the Peter Bent Brigham Hospital for the first time June 9,

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1. Longcope, W. T.: Am. J. M. Sc. **124**:209, 1902.
2. Neukirch, P.: Ztschr. f. Hyg. u. Infektionskr. **85**:103, 1918.
3. Harvey, A. M.: Arch. Int. Med. **59**:118, 1937.
4. Bornstein, S.: J. Immunol. **46**:439, 1943.
5. Seligmann, E.; Saphra, I., and Wassermann, M.: Am. J. Hyg. **38**:226, 1943.

1944. Three days before admission she vomited violently, experienced nausea and began to have severe diarrhea. These symptoms persisted until she entered the hospital. No blood was noted in the yellow vomitus or the stools. Her temperature was reported to have been as high as 104 F.

No history of having used well water or infected milk was elicited. However, many friends at her place of work had recently had diarrhea. The past history was noncontributory.

On admission she appeared a moderately ill, slightly obese woman. Her temperature was 100 F.; the pulse rate, 80; the respiratory rate, 20, and the blood pressure, 115 systolic and 90 diastolic. The abdomen was distended and tympanitic. No fluid wave was obtained. The spleen was not felt. The examination disclosed no other significant abnormalities. A rash was not observed.

The significant laboratory findings included that of S. suipestifer in a stool culture on admission. On the fifth hospital day S. suipestifer was grown from the urine. The following day S. suipestifer was grown from the blood. At first the urine and blood cultures had failed to yield S. suipestifer. Other laboratory data of importance were: a leukocyte count of 5,250 to 5,500 per cubic millimeter, with a differential count of 52 per cent neutrophils, 14 per cent band forms, 14 per cent lymphocytes and 20 per cent monocytes on the day of admission. The erythrocyte count was 4,890,000 and the hematocrit value 44 per cent. The urine contained hyaline and granular casts, protein (2+), also acetone (1+) and sugar (1+). A guaiac test of the stools was positive. Amebas were not found.

Treatment consisted primarily of parenteral administration of fluid. The temperature ranged from 99 to 101 F., with a high of 102 F. on the third hospital day. The pulse rate also remained elevated, rising as high as 120. Thirty-six hours before death, the patient rapidly became comatose, and pulmonary congestion developed. She died on the eighth hospital day.

It should be recorded that culture of a stool from the patient's husband yielded S. suipestifer.

Autopsy (three hours after death).—The body was that of a well developed, obese, elderly white woman. The skin was white and turgid. No lesions of the skin were discovered. The peritoneum, both parietal and visceral, was smooth, grayish pink and glistening. No free fluid was encountered, and there was no exudate. There was only slight congestion of the serosal vessels of the descending colon. The stomach was distended with fluid. The mesenteric lymph nodes were not enlarged.

One hundred cubic centimeters of clear brownish fluid was present in both the right and the left pleural cavity. The heart weighed 270 Gm. and presented no gross abnormalities. The right lung weighed 560 Gm. and the left 390 Gm. The lungs were moderately voluminous, and the pleural surfaces were glistening. On section the lower lobes, particularly that on the right, appeared congested and somewhat edematous. The spleen weighed 100 Gm. It was of normal consistency. The pulp was dark reddish purple. The malpighian corpuscles were indistinct.

The most important findings were those in the alimentary tract. The esophagus was freed with difficulty from the mediastinal tissues. The lumen of the esophagus was narrowed and filled with cloudy reddish gray material. The mucosal surface appeared granular and bright red to reddish black. The discoloration ended abruptly at the gastroesophageal junction. The normal mucosal pattern of the stomach was well preserved. A 3 mm. wide triangular bright red ulcer,

which appeared to have burrowed through the mucosa, was located along the lesser curvature. Approximately twenty minute irregular similar ulcers were observed in the pyloric antrum. The mucosa in the first portion of the duodenum resembled that of the esophagus. The mucosa of the remainder of the small intestine was edematous and dusky pink to pale pink. Ulcers were not seen. Peyer's patches could not be identified.

Conspicuous shallow ulcers were visible in the dependent portion of the cecum and in the ascending and the transverse colon. Some were confluent, but the majority were discrete. They were oval or somewhat irregular in shape. Occasionally they were circinate. They measured 0.5 to 3 cm. in length and 0.3 to 1 cm. in width. The arrangement in the main was parallel with the long axis of the colon. The craters were pale yellow to yellowish brown, and they were not covered with purulent exudate. The surrounding mucosa was occasionally edematous and yellow to brownish red.

The liver weighed 1,600 Gm. and was of fairly firm consistency. The surface was pale brown to brownish yellow, and the smooth sectioned surfaces were pale brown. No abscesses were encountered in many sections.

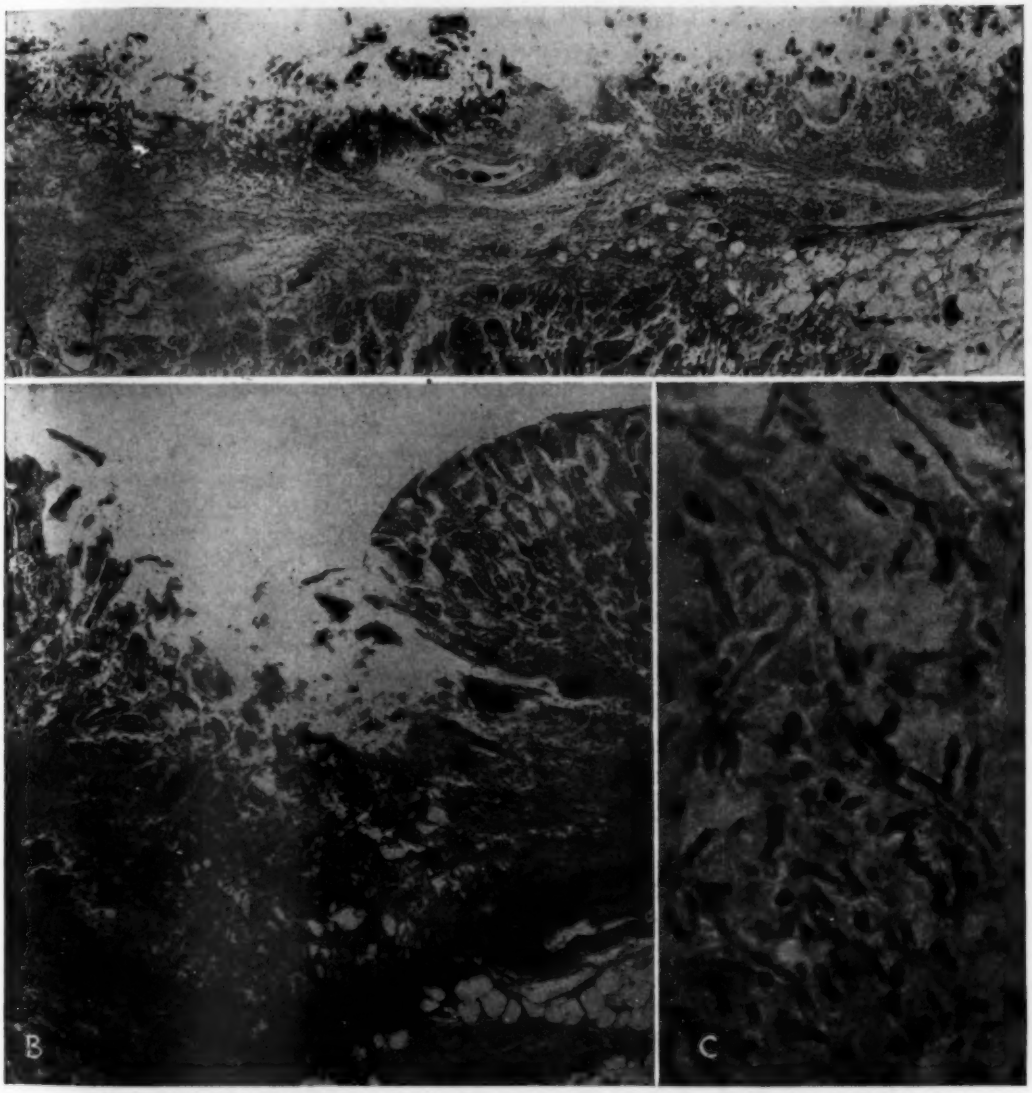
The kidneys, the adrenal glands, the bladder and the pelvic organs presented no unusual features. Tissue blocks were fixed in Zenker's solution and in Zenker's solution to which solution of formaldehyde U. S. P. had been added in the concentration of 5 per cent, instead of acetic acid. Sections were stained by the eosin-methylene blue and Gram methods.

The microscopic features of particular interest were observed in the gastrointestinal tract. The esophagus showed both mucosa and submucosa destroyed, with only fragmented strands of brown or blue necrotic material remaining. A few necrotic esophageal glands were still recognizable. It was in this necrotic material that a large number of organisms resembling monilias were found. These were gram-positive rods ranging in length from 18 to 20 microns approximately. Mycelial strands and spores were seen. A few poorly preserved blood vessels were invaded by the monilias. There was little or no cellular response around the fungous elements. The connective tissue between the muscle bundles was edematous and displayed markedly congested blood vessels. This loose stroma exhibited a scattered, primarily monocytic infiltration with only rare polymorphonuclear leukocytes. Occasional fibroblastic proliferation was recognized. The muscularis itself was not necrotic. Fibrin networks had become deposited in the adventitia of the esophagus, particularly near the gastroesophageal junction. Here, occasional monilias were seen. Only little monocytic infiltration was observed in the fibrin network. The destruction of mucosa terminated abruptly at the gastroesophageal junction (*A* in figure).

In the stomach the ulcers observed grossly proved to be acute and deep, extending down into the lamina propria (*B* in figure). The margins were slightly overhung by the intact gastric mucosa. The bases of the ulcers were composed of a thick layer of mycelial threads with accompanying spores (*C* in figure). Several blood vessels at the bases of the ulcers contained thrombi which were invaded by the monilias. Again, leukocytic response surrounding the ulcers was scant and predominantly monocytic. The muscularis was edematous. Sections taken from the gastroduodenal junction showed a process essentially similar to that shown by the esophagus, though somewhat more pronounced.

The small intestine displayed diffuse, though moderate, monocytic, lymphocytic and plasma cell infiltration, occasionally with eosinophilic polymorphonuclear leukocytes in the edematous lamina propria. The mucosa was intact, and monilial

elements were not observed. In the colon large acute ulcers were found. These were shallow, barely penetrating the lamina propria. They were sharply limited on either side by intact mucosa, which was not undermined. The bases of the ulcers were generally covered by a thin layer of fibrin enmeshing gram-negative bacilli. In many zones closely packed monocytes were seen. Only occasionally were polymorphonuclear leukocytes seen infiltrating just beneath the bases of the



A, ulceration and edema of the esophageal mucosa at the cardiac orifice. Eosin and methylene blue; × 26. *B*, acute ulceration of gastric mucosa. Eosin and methylene blue; × 37. *C*, monilial elements at the base of the ulcer shown in B. Eosin and methylene blue; × 900.

ulcers. Several vessels and lymphatics were completely or partially filled with thrombi. One vessel situated at the base of one of the ulcers exhibited early thrombus formation. Some lymphatics contained polymorphonuclear leukocytes. The leukocytic response, mostly confined to the superficial zones of the strikingly edematous lamina propria, was primarily one of large monocytes with pale cytoplasm. The mucosa and the muscularis were compressed. A few vessels in the lamina propria showed fibrinoid degeneration. There was no abnormal degree of leukocytic infiltration in the intact mucosa.

The heart showed a few foci of lymphocytes beneath the pericardium. The lungs exhibited a patchy acute exudate, most pronounced and occasionally confluent around some of the bronchioles. The inflammatory cells were for the most part neutrophilic polymorphonuclear leukocytes. Almost invariably the exudate was centered around prominent colonies of gram-positive cocci. Occasional cocci were engulfed by monocytes. In addition, strands of monilias and spores were seen in the alveoli, generally in conjunction with the cocci. Monilias were not seen intracellularly. The lumens of the bronchioles contained similar exudate and micro-organisms. The cellular masses within the bronchioles in many instances were embedded in hyaline amorphous aggregates, which were interpreted as representing aspirated material. The alveolar capillaries were considerably congested, and there was evidence of edema in the alveolar spaces. The bronchiolar walls were infiltrated by monocytes and polymorphonuclear lenkocytes.

The pulp of the spleen was markedly congested. There was only little lymphoid depletion. No bacteria were seen. The liver cells showed considerable fatty vacuolation, particularly at the periphery of the lobules. The Kupffer cells in the sinusoids were prominent. Neither bacteria nor monilial elements were seen. In the kidney, a few hyaline casts were present in the collecting tubules, and several foci of monocytes and lymphocytes were encountered in the pelvic fat. The renal parenchyma was otherwise not remarkable. Again, bacteria could not be identified. The adrenal gland exhibited considerable lipoid depletion. In the medulla minimal round cell infiltration was detected. The remainder of the sections, taken from the pancreas, the left ureter, the bladder, the uterus and the ovary, showed no significant changes.

S. suipestifer was isolated from the ileum, the colon and the blood in the heart post mortem. The H antigen of organisms obtained from the heart's blood and the colon was destroyed by heating at 100 C. for fifteen minutes. The O antigen was tested against S. suipestifer and Salmonella aertrycke stock serums. Agglutination was positive in a dilution of 1:80 against S. suipestifer serum and questionably positive against S. aertrycke serum in a dilution of 1:10.

COMMENT

The lesions in the colon, which can be presumed to be the result of S. suipestifer infection of the gastrointestinal tract, are probably of secondary importance in this case, since one is dealing with a blood stream infection. S. suipestifer is an exceedingly invasive organism. In Seligmann's [5] series the blood cultures in 60 per cent of the cases of S. suipestifer infection were positive. The organism is prone to invade other organs, as is emphasized by many different reports, only a few of

which are cited: involvement of the lungs,[6] the endocardium [7] and the meninges.[8] There may be involvement of the genitourinary tract, such as perinephric abscess,[9] and even such lesions as subdiaphragmatic abscess [10] and salpingitis.[9] For further reports see Seligmann and co-workers.[5] It should be remembered, however, that S. suipestifer infection is characterized by bactermia, thus bearing a close resemblance to typhoid. The site of invasion appears to be the gastrointestinal tract. Chronically infected human carriers of Salmonella have been reported.[4]

The colonic ulcers in this case had the appearance of relatively mild lesions. The lamina propria was barely penetrated, and the mucosa between the ulcers was intact. Conspicuous, however, was the marked edema of the submucosa, which was found to a lesser degree in the small intestine. Other noteworthy features were the predominantly monocytic and lymphocytic infiltration at the bases of the ulcers and the scant amount of fibrin deposition. The question might well be raised at this stage: Does S. suipestifer behave like Eberthella typhosa, and does it exercise a so-called negative chemotaxis as was shown to be the case for antigen prepared from E. typhosa by Morgan and Upham [11]? The low white cell count in this case with only 52 per cent neutrophils lends plausibility to this speculation, as does the statement by Harvey [3] that unless localization of the infectious process should occur, the white cell count remains at a low level. Similarly, the white cell count in the cases studied by Jager and Lamb [9] generally ranged between 5,000 and 10,000.

S. suipestifer falls into group C of paratyphoid bacilli, according to the classification of the New York Salmonella Center.[5] Gastrointestinal symptoms and lesions appear to be preeminently caused by Salmonella typhi murium, belonging to group B.[5] Angrist, in discussing a report by Bornstein,[12] described lesions somewhat resembling typhoid in a 21 year old patient who entered the hospital with signs and symptoms of appendicitis and later succumbed to peritonitis. The white cell count, however, was only 2,100. S. typhi murium was recovered from the stool and from a colonic ulcer. Peyer's patches were somewhat superficially ulcerated, hemorrhagic and packed with large mononuclear cells. Numerous small ulcers and an occasional large ulcer were found in the colon. Ulcers caused by paratyphoid A and B organisms are illustrated in an early report by Dawson and Whittington.[13] They found the lower 2 feet (61 cm.) of the ileum constantly affected with deep punched-out ulcers and swelling of the lymphoid follicles. The large intestine was severely involved in many instances.

6. Shaw, F. W.: J. Lab. & Clin. Med. **12**:141, 1926. Cole, D. B., and Nalls, W. L.: ibid. **23**:1223, 1938.

7. Goulder, N. E.; Kingsland, M. F., and Janeway, C. A.: New England J. Med. **226**:127, 1942.

8. Neter, E. R.: Arch. Int. Med. **73**:425, 1944.

9. Jager, B. V., and Lamb, M. E.: New England J. Med. **228**:299, 1943.

10. Hunter, J. E.; Andersen, C. A., and Hutchinson, W. B.: Northwest Med. **43**:114, 1941.

11. Morgan, H. R., and Upham, H. C.: Proc. Soc. Exper. Biol. & Med. **48**:114, 1941.

12. Bornstein, S.: New York State J. Med. **42**:163, 1942.

13. Dawson, B., and Whittington, T. H.: Quart. J. Med. **9**:98, 1915-1916.

Morphologically, there is little to suggest an overwhelming toxemia in the present case. The Kupffer cells, it is true, were somewhat prominent. However, the spleen, unlike that affected by typhoid, which is characteristically enlarged and soft, failed to show anything resembling a "splenic tumor." Therefore, Bornstein's [4] previous statement with regard to absence or nonspecificity of lesions in Salmonella septicemia is not refuted by the histologic observations in this case.

The importance of the colonic ulcers is overshadowed by the severe esophageal ulcers, the necrotizing lesions in the duodenum and the pinpoint ulcers in the stomach. Fungi having the morphologic characteristics of Candida albicans or monilias were present in large numbers. Mycelial threads were lying within the necrotic esophageal and duodenal mucosa, surrounded by spores. In the stomach the organisms formed a thick layer at the bases of the ulcers. Thrombus formation within the vessels at the ulcer base was a noteworthy finding. The leukocytic response to the monilias was primarily monocytic and lymphocytic, with slight fibroblastic proliferation. This was similar to the cellular response in the ulcers of the colon. Thus the tissue reaction appeared to be of some standing but was without resemblance to a granulomatous process. The presence of monilias in the lung, together with numerous gram-positive cocci and an acute inflammatory reaction largely around and also to some extent within bronchioles, was consistent with aspiration pneumonia.

C. albicans proliferates in the tissues as mycelial threads, accompanied by budding or nonbudding ovoid forms. According to Swartz,[14] six species can be identified in freshly isolated cultures. Castellani [15] attempted differentiation by biochemical methods. The virulent strains cause death of rabbits a few days after intravenous injection. The manifestations of moniliasis are manifold; one of the most common is the oral thrush of childhood. Benham and Hopkins [16] recovered C. albicans from the alimentary tracts of 18 of 100 apparently normal young adults. Langenbeck first described C. albicans as a human pathogen in 1839. He found the fungus in patches of thrush on the oral mucosa, the pharynx and throughout the intestinal tract in a patient who had died of typhoid.[14] This early observation has a definite bearing on the pathogenic properties of monilias. C. albicans is prone to attack debilitated persons or to be a secondary invader. Lewis and Hopper [17] stated that "there is more or less corresponding increase with age in the involvement of the gastrointestinal tract" without further amplification. Yet the condition seems to be an uncommon finding, or it is possibly overlooked at autopsy.

In a series of 5,059 consecutive autopsies from 1913 to 1943 at the Peter Bent Brigham Hospital only 3, according to the records, revealed esophageal thrush. It is noteworthy that all 3 patients were suffering

14. Swartz, J. H.: Elements of Medical Mycology, ed. 1, New York, Grune & Stratton, Inc., 1943, pp. 45-62.

15. Castellani, A.: Fungi and Fungous Diseases. Chicago, American Medical Association, 1928, p. 24.

16. Benham, R. W., and Hopkins, A.: Arch. Dermat. & Syph. **28**:532, 1933.

17. Lewis, G. M., and Hopper, M. E.: An Introduction to Medical Mycology, ed. 1, Chicago, The Year Book Publishers, Inc., 1939, p. 129.

from debilitating diseases: Two had diabetes; one of these had pulmonary tuberculosis, and the other had marked arteriosclerosis with gangrene. The third patient had ulcerative colitis. Severe necrosis of the esophageal mucosa was present in 2 of the patients, while in the third the lesions were less pronounced. Polymorphonuclear leukocytes were more numerous in these lesions than in those found in the present case, although monocytes were also abundant. The case presented here bears a striking resemblance to 1 of the cases described by Irish.[18]

In children, on the other hand, thrush esophagitis is not an uncommon clinical entity. Ebbs [19] reported 28 cases of esophagitis in infancy; 22 proved to be due to C. albicans. Reye [20] described 5 cases of esophagitis due to Monilia albicans encountered in 150 autopsies performed on infants. The pathologic observations appear to be similar to those in the present case.

Several further points of interest are to be noted in the history. 1. The patient died in June. Salmonella infection is most common in the hottest months of the year, with a peak in September.[5] 2. There was a history of vague gastrointestinal disturbances of companion workers; S. suipestifer was grown from the stool of the patient's husband. Although S. suipestifer infections generally sporadic, there appears to be some epidemiologic background in this instance. However, no proof is available, and the hypothesis of cross infection must remain mere speculation. 3. Mortality of adults due to S. suipestifer infection is high.[21]

SUMMARY

A 60 year old woman was afflicted with a severe gastrointestinal disturbance and finally succumbed to the septicemia of S. suipestifer. The colonic ulcers probably cannot be considered responsible for the death of the patient. The severe esophagitis, the gastric ulceration and the duodenitis caused by C. albicans infection, shown on pathologic study, were unsuspected during life. In this instance the fungus is considered a secondary invader in a debilitated patient.

18. Irish, R. H.: New York State J. Med. **36**:1491, 1936.

19. Ebbs, J. H.: Arch. Dis. Childhood **13**:211, 1938.

20. Reye, D.: M. J. Australia **2**:673, 1941.

21. Keefer, C. S.: New England J. Med. **222**:105, 1940.

PRIMARY CARCINOMA OF THE LIVER OF A DOG

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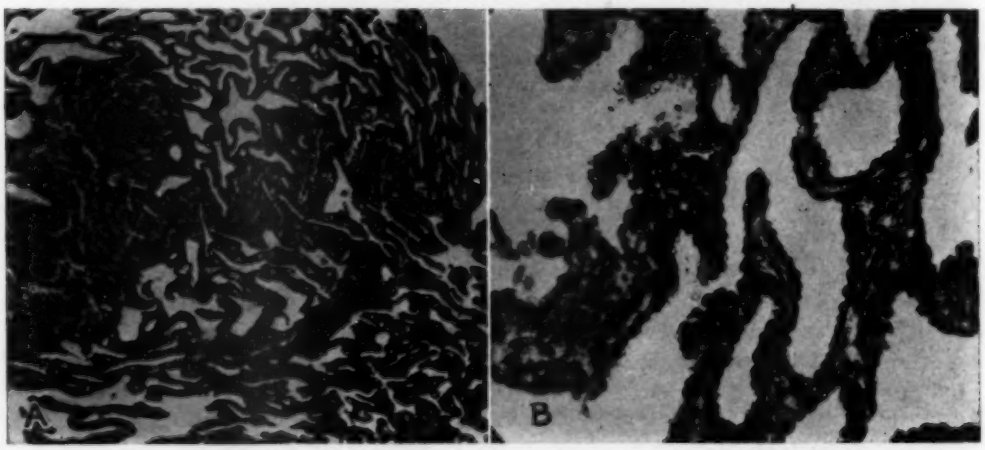
IN SPITE of the large volume of work on cancers of animals, both those experimentally produced and those incidentally observed, primary carcinoma of the liver is rare in dogs. It is well known that the incidence of primary carcinoma of the liver is low also in the human race of this country and of Europe. Goodpasture [1] has shown that in dogs there is some relation between the incidence of tumors and senescence. Even so, the incidence of primary carcinoma of the liver in the senile animals studied by Goodpasture was low. Carcinoma of



A, section of carcinoma of the liver under low power magnification, showing the proliferating epithelium forming a marked papillary pattern and pseudoacinous production.

B, section of carcinoma of the liver under high power, showing the tumor composed of proliferating bile duct epithelium with a supporting stroma of connective tissue. In the upper half are masses of hyperchromatic cells in which the papillary pattern is lost.

the liver (not experimentally produced) has been reported observed in rats and fowl.[2]

From the departments of pharmacology and pathology, Howard University School of Medicine.

1. Goodpasture, E. W.: J. Cancer Research **1**:363, 1916.

2. Kahlau, G.: Frankfurt. Ztschr. f. Path. **50**:361, 1937. Itami, S.: J. Cancer Research **3**:275, 1918.

While making glycogen determinations on the livers of dogs in connection with another problem, the carcinoma herein reported was seen, and in view of its rarity we regarded it as being worthy of reporting. We make no claim that this is an initial contribution.

REPORT OF A CASE

An old female dog, weighing 15.5 Kg., somewhat decrepit and having cataracts, had been under prolonged pentothal sodium anesthesia the day before it was killed. When the liver was removed, a large mass was observed on the dorsal aspect of the large right lobe. Careful examination failed to show a tumor in any other organ or tissues.

The liver and the gallbladder together weighed 550 Gm. The external and the cut surfaces were reddish brown, and the consistency was firm. Protruding from the dorsal surface of the large right lobe of the liver there was a soft, whitish ovoid tumor that had a greater diameter of 3 cm. and a lesser diameter of 3.4 cm. It was circumscribed but not encapsulated.

Microscopically the tumor was composed of glandular tubules, varying in size and in shape, supported by a stroma of connective tissue, forming a papillary pattern. The acini were lined with a single layer of cuboidal cells with deeply staining nuclei. In some fields, however, the epithelium was thickened by masses of anaplastic cells that projected into the lumen. Mitotic figures were not frequent. There was no sharp line of demarcation between the tumor and the hepatic tissue. Some isolated glands, surrounded by hepatic parenchyma, were seen at variable distances from the main tumor. The liver cells did not show significant histologic changes (figure).

COMMENT

In a recent paper Mulligan [3] called attention not only to the low incidence of primary carcinoma of the canine liver but to the small number of reports that have been made on canine neoplasms. He has made a plea to veterinary hospitals for more routine autopsies and reasonably detailed reports of them. We believe that the plea should be extended to laboratory workers, who should at least grossly examine the organs of dead experimental animals to determine whether or not tumors are present. If this is done on a wide scale, we believe the statistics on tumors occurring in dogs and other animals will be more extensive.

3. Mulligan, R. M.: Arch. Path. **38**:115, 1944.

HEMOPERICARDIUM FROM PERICARDIAL METASTATIC CARCINOMA

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DEATH from bleeding into the pericardial sac may be sudden and unexpected. Most frequently this occurrence is associated with a tear of the aorta within the pericardial sac or with cardiac rupture in a patient suffering from myocardial damage. Blood in quantities sufficient to tint the pericardial exudate is occasionally present in pericarditis associated with pneumonia and uremia. Bleeding into the pericardial sac from metastatic tumor growths in amounts capable of causing cardiac constriction and death is unusual. A case history recording such an event follows.

REPORT OF A CASE

A painter 45 years of age, who had been a champion soccer player, entered the Loretto Hospital, June 10, 1945, for treatment of typical lobar pneumonia of the left lung and pleurisy. The presence of these conditions was confirmed by roentgen studies, which also revealed marked fibrosis throughout the right lung. The patient was given a series of intramuscular injections of penicillin (350,000 units) and made a satisfactory recovery.

Within two weeks, however, he was again stricken with pneumonia and was treated as in the first attack. Thereafter he had difficulty in breathing and his respiration was noisy.

On July 20 he again became ill and had foul sputum containing a few fusiform bacilli, a few Vincent's spirochetes and many staphylococci. Moist rales were heard in both lungs, and the liver was enlarged and tender. It was thought the fingers showed slight clubbing, and a diagnosis of cor pulmonal was made. The patient was treated with digalen. He had considerable difficulty in breathing and complained of substernal pain. During the two days of his terminal hospital stay, cardiac and respiratory stimulants failed to relieve the thoracic pain although they increased the blood pressure. On one occasion the attending physician thought that the patient was made worse by such therapy. The patient died about an hour later.

At autopsy the body was powerfully developed, was 6 feet tall and weighed 220 pounds (100 Kg.). The left pleural cavity contained 500 cc. of clear yellow liquid, and the left lung was involved in atelectasis. The right pleural cavity was obliterated by fibrous tissue, and the right lung revealed primary bronchiogenic carcinoma of the bronchus to the upper lobe. The tumor extended along the bronchus about 22 mm. and encircled about 75 per cent of the circumference. It obstructed about one third of the lumen and was firm and yellow-gray. There was extensive involvement of the surrounding parenchyma in the form of clusters of metastases, and similar growths occurred in the tracheobronchial lymph glands.

The pericardial sac contained about 160 cc. of liquid blood and about 45 Gm. of soft red-brown clot. The latter was in part attached to the epicardium, but much of it was in an irregularly ridged mass in the sac. The source of bleeding was an irregular clump of carcinomatous nodules on the epicardium close to the interventricular septum. The individual masses were 2 to 11 mm. in

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diameter, and two had oozing zones of hemorrhage as much as 0.5 mm. in diameter. The heart weighed 480 Gm. and had a normal arrangement of valve leaflets, chambers and vascular trunks.

The patient's father died of carcinoma of the stomach at 47 years of age, while his mother, aged 70 years, was suffering from carcinoma of the uterine cervix. A brother died when 26 years old, of pulmonary tuberculosis.

SUMMARY

In a case of fatal bleeding into the pericardial sac from epicardial carcinomatous metastases, the primary tumor, an epidermoid type of bronchiogenic carcinoma, was in the upper lobe of the right lung. The metastases reduplicated the original growth and were highly vascular. Several had bleeding regions, the blood from which, accumulating in the pericardial sac, caused cardiac compression and death.

TERATOMA OF PINEAL GLAND WITH CHORIOCARCINOMA AND RHABDOMYOSARCOMA

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IN THIS report we shall record the clinical and pathologic observations made in a case of pineal teratoma in which choriocarcinoma and rhabdomyosarcoma were found. Askanazy,[1] van den Berg and co-workers[2] and Russell[3] have reported similar cases. Bochner and Scharff[4] analyzed the recorded cases and added 3 of their own in 1938, and they discussed the various types of tissue found. Additional cases were reported by Lichtenstein,[5] Gerstley[6] and Russell.[3] Throughout the literature on the tumor generally designated as "pinealoma" runs a controversy as to whether it is a tumor of the pineal cells or a teratoma. In the most recent article Russell concluded that in most of the instances the tumor was teratomatous, but he expressed the belief that in an occasional one the tumor may be true pinealoma. The resemblance of the tumor known as "pinealoma" to "seminoma" and "embryonal carcinoma" of the testicle and to "dysgerminoma" of the ovary is quite suggestive of a relationship.[7]

The desirability of investigations of the urinary gonadotropic hormone in patients with symptoms of pineal tumor has been suggested.[4] This case further emphasizes this possibility.

REPORT OF A CASE

A 17 year old youth was admitted to the Indiana University Medical Center July 28, 1936. He complained chiefly of headache, vomiting, turning inward of each eye, and staggering on walking. His present illness had commenced about four weeks before admission with involuntary elevation of the eyes and headache. He then began to stagger a little on walking. Four days before coming to the hospital his eyes "turned in." At this time projectile vomiting developed. His previous medical history was essentially irrelative. He had always been thought to be quite retarded mentally.

Neurologic examination showed him to be in a semistuporous state. The skull was normal. The pupils were dilated and equal, and did not react to light. There was a marked convergence spasm of the eyes. He was unable to elevate or depress them.

From the Departments of Neurosurgery and Clinical Pathology, Indiana University School of Medicine.

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2. Hijmans; van den Berg, A. A., and van Hasselt, J. A.: Nederl. tijdschr. v. geneesk. **1**:1271, 1913.

3. Russell, D.: J. Path. & Bact. **56**:145, 1944.

4. Bochner, S. J., and Scharff, J. E.: Arch. Surg. **36**:303, 1938.

5. Lichtenstein, B. W.: Arch. Neurol. & Psychiat. **44**:153, 1940.

6. Gerstley, J. R.: J. Pediat. **17**:512, 1940.

7. Harris, W., and Cairns, H.: Lancet **1**:3, 1932.

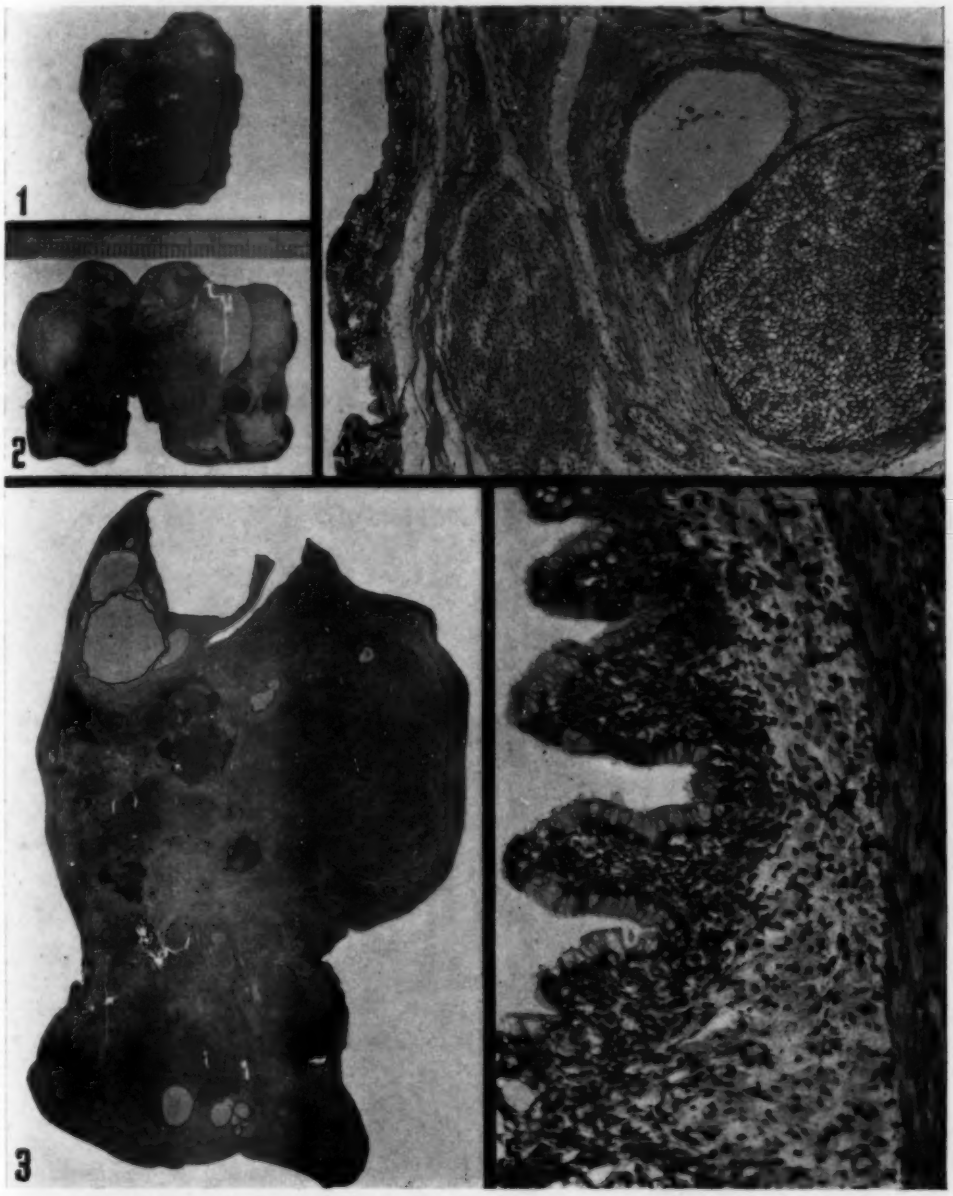

Fig. 1.—Tumor as removed.

Fig. 2.—Tumor opened in sagittal plane.

Fig. 3.—Sagittal section of tumor.

Fig. 4.—Low power magnification of a cystic portion of the tumor showing cysts lined with mucus-secreting epithelium and a mass of cartilage in the connective tissue septum.

Fig. 5.—High power magnification of a cyst wall showing smooth muscle, submucous connective tissue and columnar mucus-secreting epithelium.

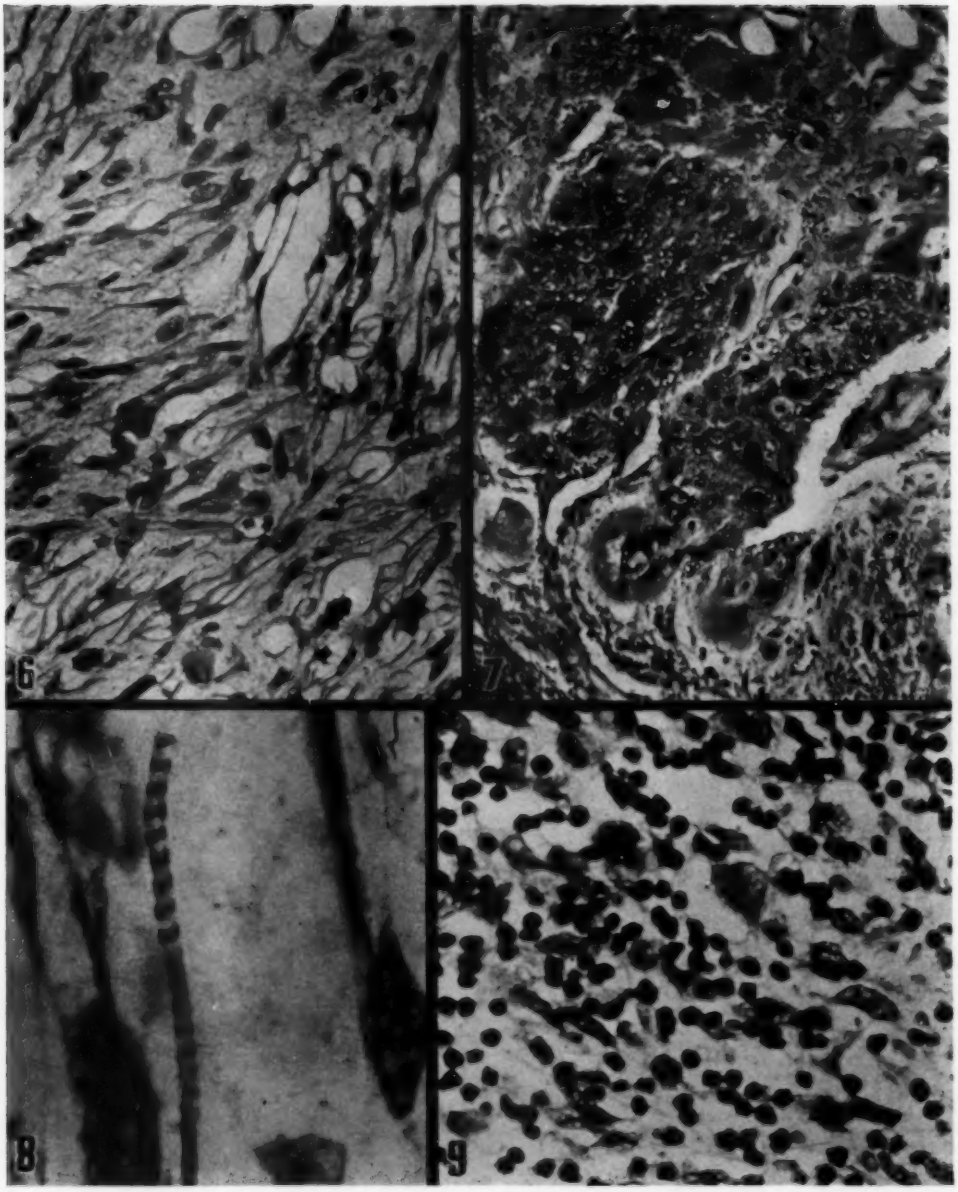

Fig. 6.—Rhabdomyosarcoma (hematoxylin and eosin; high power).
Fig. 7.—Choriocarcinoma (high power).
Fig. 8.—Striated muscle fiber (Heidenhain's iron-hematoxylin; oil immersion).
Fig. 9.—Area resembling classic "pinealoma" (high power).

There was bilateral papilledema of about 4 diopters. The lower part of the left side of the face and the left limbs seemed to be a little weak. The Babinski reflex was positive on the left. The neck was slightly rigid. In all other respects the examination gave negative results.

A general examination showed nothing of special note. The genitalia were of the normal adult male type. The urine and the blood were normal. The Wassermann, Kahn and Kline tests were negative. Roentgenograms of the skull showed some irregular calcification in the region of the pineal gland.

Ventriculography was performed August 1, about 100 cc. of fluid being removed and replaced with air. The roentgenograms showed a large filling defect in the posterior half of the third ventricle, apparently caused by a pineal tumor. The anterior half of the third ventricle and both lateral ventricles were dilated.

August 5 a transventricular approach to the tumor was attempted unsuccessfully after turning down a right parieto-occipital osteoplastic flap. A generous decompression was made at the base of the flap, and the incision was closed. August 17 it was noted that the convergence spasm was considerably less marked and that the papilledema had receded to about 2 diopters in each eye.

August 24 the osteoplastic flap was reelevated and the inner half of the right occipital lobe resected. A tumor about the size of a golf ball was found just beneath the splenium of the corpus callosum. It was yellowish gray, firm in consistency, irregularly nodular and well encapsulated. It was separated carefully from surrounding structures and removed in one piece. The bone flap was replaced and the incision closed.

His general condition was satisfactory the afternoon and evening of the day of operation. The following morning he had an attack of opisthotonos. A spinal puncture was performed, bloody fluid being obtained. Successive attacks of opisthotonos occurred. His general condition became progressively poorer. Death occurred August 27.

Gross Specimen.—The tumor was ovoid, 33 by 31 by 30 mm. The outer surface was generally rough and hemorrhagic except for a rounded glistening area at one pole overlying a cystic portion. On cut section three types of tissue were found. One was composed of small cysts; another, of solid white firm tissue, and the larger part, of red, granular, seminecrotic tissue. Some cysts contained clear fluid; others, mucus.

Microscopic Examination.—A sagittal plane section showed several epithelium-lined cysts. Some were lined with mucus-secreting columnar epithelium resembling intestinal mucosa; others showed ciliated epithelium resembling respiratory mucosa. The white firm area of the tumor was made up of irregular spindle-shaped cells of an immature type which on further study proved to be rhabdomyosarcoma; cross striations were demonstrated in the fibers of the cytoplasm of the spindle-shaped cells. The hemorrhagic areas of the tumor were necrotic except for a few islands of tissue present which exhibited masses of syncytial cells in conjunction with proliferating Langhan's cells. A very small area was suggestive of the usual "pinealoma." This area merged almost imperceptibly into the area of sarcoma. Spherules of calcium were found in the myosarcoma and in the hemorrhagic tissue of the choriocarcinoma.

Autopsy.—The autopsy showed no significant additional features. The tumor had been completely removed. No pineal tissue was found. The autopsy included the abdomen and the thorax. Unfortunately, neither the testicles nor the prostate were examined microscopically. The lungs showed patchy pneumonia and no metastasis.

Laboratory Methods and Technical Notes

A PORTABLE EXHIBIT CASE

GEORGE J. RUKSTINAT, M.D., CHICAGO

THE NEED for a portable case in which to exhibit Translite photographs, to supplement the usual demonstration shelf for preserved specimens in the pathologic museum, prompted the designing of the device described here. The general utility has been expanded to include its being used in classrooms and clinics and at conventions of medical societies.

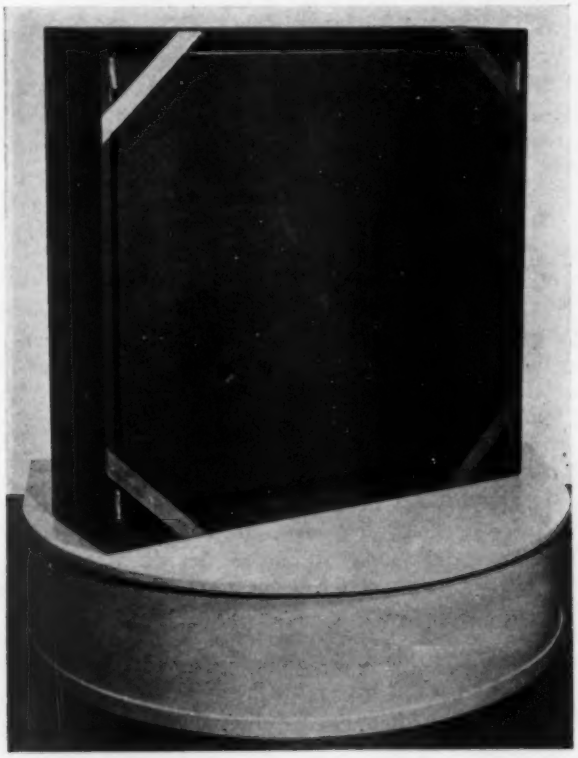

Fig. 1.—The case packed for shipping, showing a rear view.

Basically the case is a sturdy sectional contrivance, constructed of readily available materials. The boxlike base is 42 inches (106.5 cm.) high and wide. Its front is common plywood, ⅜ inch (about 1 cm.) thick, and the sides, the

From the Department of Pathology, Presbyterian Hospital.

top and the bottom are pine, ⅞ inch thick and 12 inches wide (about 2 cm. thick and 30.6 cm. wide). The back is open so that it can receive the top when the case is to be transported. Four metal braces 8 inches by 1½ inches by ⅛ inch (about 20 by 4 by 0.3 cm.) reenforce the corners in back and are screwed to the sides and the top or the bottom (fig. 1) and to the viewing top when packed for shipping. They lend rigidity to the structure, keep the top safely within the base and provide a grip for carrying.

The viewing case is 38½ inches wide and high and 10⅞ inches deep (about 97.5 and 27.5 cm.). Its sides, top and bottom are of pine ⅞ inches thick. The front is made of a hinged and locked window sash with twelve lights, 10 inches high and 8 wide (about 25.5 and 20 cm.) (fig. 2). The sides and the top of the

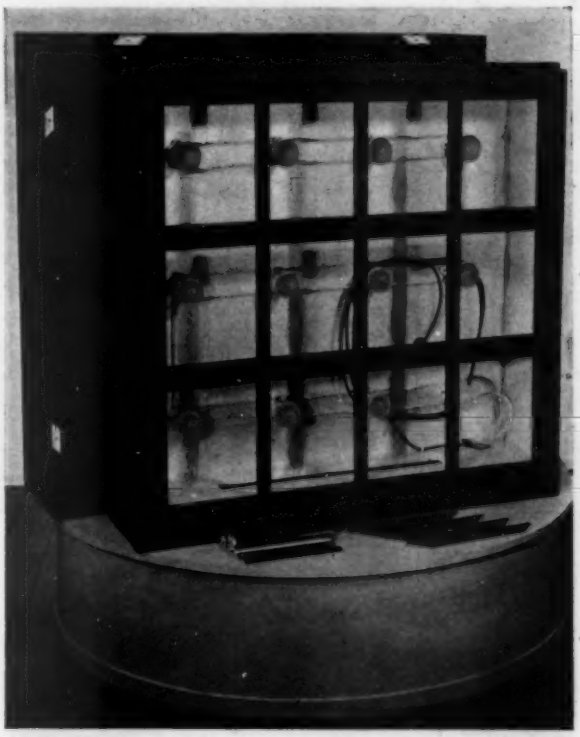

Fig. 2.—Front view of top section, showing arrangement of lights and film spaces.

sash are 2½ inches wide and the bottom is 3½ inches wide (about 6 and 9 cm.). The sash is mounted so that the recesses, to receive the panes, are inside. In actual use a Translite film is mounted between two panes of single thickness glass, and the panes are bound together with gummed paper tape. The photograph is then held in place by small strips of molding screwed to the sash (fig. 3). Descriptions of the films are made on 6 inch by 1 inch (14 by 2.5 cm.) cards slipped into a holder made up of two strips of molding under each.

The inside of the case was given two coats of aluminum paint to enhance reflection from the twelve lights which are mounted on the back and centered so

that each will primarily light one photograph in the front. The diffusion from other lights minimizes spot lighting of the film. The lights are common Mazda lamps of 60 watts each, set in porcelain bases, which are connected in parallel by asbestos-insulated wire.

Escape of heat is provided for by twenty-four holes ½ inch (about 1 cm.) in diameter, which were drilled into the top at a 45 degree angle to minimize escape of light and entrance of dust. The bottom is set up 2 inches (5 cm.) from the lower ends of the sides in order to provide a safe recess for the switch which controls the lights and also to allow a space for the circulation of air. An opening for air in the bottom is 2 inches by 20 inches (5 cm. by 51 cm.), placed as a long slit near the middle.

Fig. 3.—A close-up view of the interior of the viewing section, showing molding cleat to retain film.

The back of the viewing box is made of ⅜ inch plywood and contains a single 2 inch hole through which the electric plug and wire connection to a current source can be retracted into the case during shipment. This back also protects the lighting connections and glassed sash during shipment (fig. 1). The outside of both base and viewing box were given two coats of flat black paint. Together they weigh 93 pounds (42 Kg.), the top alone, 54 pounds (24.5 Kg.). As used at present, the top is simply set on the base and plugged into the electric current. Mounted on casters and permanently assembled, the case can serve many departments, and the base can be used to store a variety of exhibits.

IMPROVED METHODS FOR DEMONSTRATING AMYLOID IN PARAFFIN SECTIONS

BENJAMIN HIGHMAN, M.D., BETHESDA, MD.

THE PRESENCE of amyloid in paraffin sections may be demonstrated by certain metachromatic aniline dyes. For this purpose, the deparaffinized hydrated sections are commonly stained with aqueous solutions of methyl violet or crystal violet, often followed by differentiation in dilute acids.[1] The violet red metachromatic color imparted to the amyloid by these dyes is changed to blue by such dehydrating agents as acetone, dioxane and the various alcohols which are commonly used before clearing in xylene and mounting in balsam or clarite. Hence, the hydrated stained sections are usually mounted in water, glycerin, syrup or saturated aqueous solution of fructose or potassium acetate.

In skilled hands, such staining methods are generally satisfactory. However, water, glycerin and nonsetting aqueous solutions are unsatisfactory as permanent mounting mediums. If glycerin or Apáthy's syrup is used, there is frequently noted within a short period marked fading or obscuring of detail due to diffusion or bleeding of the stain into these mounting mediums. Furthermore, nuclei are often poorly stained, and considerable care is required to prevent understaining or excessive decolorizing by the acid and to prevent overstaining or insufficient differentiation. Overstaining changes the color of the amyloid to purple or blue and lessens the contrast.

These faults can be corrected to a large extent by using the following technic:

Deparaffinize sections in xylene and pass through graded alcohols to water in the usual manner.

Stain five minutes with hematoxylin, preferably by Weigert's iron-hematoxylin-acid method, then wash in water.

Stain one to five minutes in a 0.5 per cent solution of crystal violet or methyl violet in 2.5 per cent acetic acid.

Wash in water and mount in the modified Apáthy's syrup described in a later paragraph.

This technic has been used successfully on a great variety of tissues fixed with formaldehyde. Amyloid, cartilage and certain types of mucoid material were stained purplish to voilet red against a bluish background. Nuclei were stained sharply by the hematoxylin.

From the Pathology Laboratory, National Institute of Health, United States Public Health Service.

1. (*a*) Lillie, R. D., cited by Conn, H. J., and Darrow, M. A.: Procedures Used by the Biological Stain Commission, Geneva, N. Y., Biotech Publications, 1943. (*b*) Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938. (*c*) Conn, H. J., and others: Biological Stains, ed. 4, Geneva, N. Y., Biotech Publications, 1940. (*d*) Romeis, B.: Taschenbuch der mikroskopischen Technik, ed. 13, Munich, R. Oldenbourg, 1932.

Prestaining with hematoxylin improved nuclear staining markedly. The acetic acid added to the staining solution greatly retarded overstaining without fading the hematoxylin appreciably and thus permitted a wide range of staining time. Good results were obtained by staining for periods of from as little as thirty seconds in a 0.1 per cent solution of the dye to as long as thirty minutes in a 0.5 per cent solution of the dye in 2.5 per cent acetic acid. Certain other acids, such as dilute to concentrated hydrochloric acid and formic acid, exerted a similar retarding influence on overstaining by the aniline dyes, but they caused fading of the hematoxylin after a few minutes. When 2.5 per cent oxalic acid was used in place of acetic acid, fading of the hematoxylin was rapid, but nuclear staining by the crystal violet was fair, so that the hematoxylin could be omitted if sharp nuclear staining was not required.

There was no appreciable diffusion or bleeding of the dye when saturated aqueous solutions of fructose or potassium acetate were used as mounting medium. Diffusion was quite marked in glycerin and less so in Apáthy's syrup prepared as described by Mallory[1b] or as modified by Lillie and Ashburn.[2] As modified by the latter authors this syrup consists of 50 Gm. each of acacia and cane sugar dissolved in 100 cc. of water with 1.5 cc. of 1 per cent merthiolate added as a preservative. Bleeding into this medium can be prevented or minimized by adding 50 Gm. of potassium acetate. A smaller amount of potassium acetate was less effective, and a larger amount greatly delayed the setting of this mounting medium. Certain other crystalloids such as sodium and calcium chloride, sodium acetate, ammonium nitrate or potassium citrate, exerted a similar action against diffusion of the stain when they were dissolved in the mounting medium. Recently we have been adding 10 Gm. of sodium chloride in place of the potassium acetate. Results are good, and the chloride interferes less with the hardening of the syrup than does 50 Gm. of potassium acetate. Attempts to preserve the metachromasia of hydrated stained sections in balsam or clarite have proved unavailing.

Another widely used method for demonstrating amyloid in paraffin sections is Bennhold's congo red stain[3]: The deparaffinized and hydrated sections are stained ten to twenty minutes or longer in a 1 per cent aqueous solution of congo red, dipped fifteen seconds in a saturated aqueous solution of lithium carbonate, decolorized in 80 per cent alcohol until clouds of stain no longer come from the sections, washed in water fifteen minutes, counterstained with alum-hematoxylin solution, washed in water, dehydrated in 95 per cent and absolute alcohol, cleared in xylene and mounted in balsam. The results are often excellent. However, the time consumed is rather long, and the amyloid may be largely decolorized if differentiated in alcohol more than a few minutes.

I have found the following modification quicker and better:

Stain deparaffinized sections until deep red with 0.5 per cent congo red or congo corinth G (color index no. 375) in 50 per cent alcohol. Usually one to five minutes is adequate.

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2. Lillie, R. D., and Ashburn, L. L.: Arch. Path. **36**:432, 1943.
3. Bennhold, H.: München. med. Wchnschr. **2**:1537, 1922.

Wash in water and differentiate one to three minutes in 80 per cent ethanol containing about 0.2 per cent potassium hydroxide. Rinse in water and counterstain with hematoxylin.

Wash in water, dehydrate, clear and mount by successive use of acetone, acetone-xylene, xylene and clarite.

The congo red mixture was prepared by adding an equal volume of alcohol to a 1 per cent aqueous solution of congo red. As the concentration of the alcohol in the staining mixture was increased up to 85 per cent, the time required for staining by a given concentration of dye was reduced. Perhaps this acceleration of staining is due to a relatively decreased affinity of the dye for the solvent, since the solubility of the dye in the solvent is diminished as the percentage of alcohol is increased. No marked difference in acceleration was noted between 50 per cent acetone, dioxane, methanol, ethanol, propanol, isopropanol and tertiary butanol. Alkalis, such as potassium hydroxide or disodium phosphate, were less efficient than alcohols as accelerators and when present in effective concentrations tended to loosen the sections from the slides.

In comparison with lithium carbonate followed by 80 per cent ethanol as used in the Bennhold method, 80 per cent ethanol with added potassium hydroxide differentiated the sections stained with congo red about as rapidly and decolorized the amyloid more slowly. Thus, in many series of paraffin sections the amyloid was decolorized by the Bennhold method in less than thirty minutes but retained considerable congo red even after an overnight exposure in ethanol containing potassium hydroxide. In a few series, however, the amyloid lost its color by either method within a few minutes, though somewhat more slowly in the 80 per cent alcohol containing potassium hydroxide. Decolorization was less rapid when the alcohol was more dilute.

Other substances, such as corpora amylacea of the prostate or brain, elastic fibers and some cornifying epithelial cells often retained the congo red stain for a variable period after differentiating in alcohol. No marked difference was noted between the two methods in the length of time the congo red was retained by such substances. Congo corinth G (also called Erie garnet B), which is closely related chemically to congo red, could be substituted for the latter but had no particular advantages. The sample used was lot NGe-2 of National Aniline and was labeled Erie garnet B.

Hematoxylin may be used either before or after the congo red stain. Either alum-hematoxylin or iron-hematoxylin may be used, depending on preference for nuclear color.

SUMMARY

In one of the two improved methods of staining amyloid in paraffin sections of tissues fixed in solution of formaldehyde the deparaffinized hydrated sections are stained five minutes with iron-hematoxylin to bring out nuclear detail, washed in water, stained one to five minutes in a 0.5 per cent solution of crystal violet or methyl violet in 2.5 per cent aqueous solution of acetic acid, washed in water, and mounted in Lillie's Apáthy's syrup, modified by dissolving 50 Gm. of postassium acetate or 10 Gm. of sodium chloride in 100 cc. of the syrup.

In the second method the deparaffinized sections are stained one to five minutes in 0.5 per cent congo red or congo corinth G in 50 per cent alcohol, differentiated one to three minutes in 80 per cent alcohol containing 0.2 per cent potassium hydroxide, counterstained with alum-hematoxylin or iron-hematoxylin, washed in water, and dehydrated, cleared and mounted by successive use of acetone, acetone-xylene, xylene and clarite.

Notes and News

Appointments.—Lawrence W. Smith, formerly professor of pathology in Temple University School of Medicine, Philadelphia, is now medical director of the Commercial Solvents Corporation, New York.

Colonel Raymond O. Dart, deputy chief surgeon for the Army Forces in the Western Pacific, has been appointed assistant director of administration at the Army Institute of Pathology. Colonel Dart has been awarded the Legion of Merit and the Bronze Star for his services since going to the Pacific in 1942 as commanding officer of the 105th General Hospital.

Alfred Golden, Washington, D. C., has been appointed assistant professor of pathology at the University of Tennessee College of Medicine, Memphis, Tenn., and director of laboratories at the Baptist Memorial Hospital, Memphis.

Lieutenant Colonel P. J. Melnick, Medical Corps, Army of the United States, on release from active duty was appointed associate professor of pathology at the University of Southern California School of Medicine.

L. S. King has been appointed pathologist and director of laboratories at the Illinois Masonic Hospital, Chicago.

L. C. Kress, formerly director of the cancer division of the New York State Department of Health, is now director of the Roswell Park Memorial Institute, until recently known as the State Institute for the Study of Malignant Diseases, Buffalo.

Valy Menkin, assistant professor of pathology at Duke University School of Medicine, has been appointed associate professor of experimental pathology in the newly created department of surgical research at Temple University School of Medicine in Philadelphia.

Peter Gruenwald has been appointed assistant in pathology in the Long Island College of Medicine, Brooklyn.

Awards.—Ernest W. Goodpasture, professor of pathology and dean of Vanderbilt University School of Medicine, is the recipient of the Passano Foundation Award for 1946, established in 1944 by the Williams & Wilkins Company, medical publishers, Baltimore. Dr. Goodpasture was given the award for his "original development of the method for propagation of viruses in pure culture by inoculation of chick embryos and for his outstanding contributions to advancement of knowledge of the cell parasite relationship in bacterial and virus infection."

Colonel E. R. Long, chief consultant in tuberculosis, Surgeon General's Office, has been awarded the Legion of Merit for his development of standards for the detection of tuberculosis, including his service in creating "priceless permanent files of chest x-ray films of army personnel."

Society News.—The new officers of the American Association for Cancer Research are William U. Gardner, New Haven, Conn., president; J. J. Bittner, Minneapolis, vice president; C. W. Hooker, New Haven, Conn., acting secretary-treasurer.

P. R. Cannon, University of Chicago, R. F. Loeb, Columbia University and E. R. Long, University of Pennsylvania, have been elected members of the National Academy of Sciences.

Public Health Laboratory Fellowships. — Five fellowships have been established by the New York State Department of Health for the training of physicians in the public health laboratory field. The appointments are for a period of one year and are open to physicians with postgraduate laboratory training who wish additional experience in pathology, bacteriology or biochemistry to qualify for positions in the public health laboratories in New York. Candidates must be United States citizens who have graduated from a medical school approved by the American Medical Association and who are licensed or eligible to take the examination for license to practice medicine in New York state. Inquiries should be directed to the Division of Laboratories and Research, State Department of Health, Albany 1, New York.

Children's Cancer Center.—A service to assist in the diagnosis and treatment of tumors in early life has been organized in the Medical Center for Children in Boston. The object is to give free diagnostic assistance and advice to any physician who will send the necessary data, sections and roentgenograms. The project, which includes research, is supported by a grant from the National Cancer Institute. The consultants are S. B. Wolbach, C. F. Branch and S. Farber, pathologists, and E. B. D. Neuhauser, roentgenologist, and members of the staff of the Children's Hospital.

Death.—Milton J. Rosenau, outstanding for his work in public health and preventive medicine, died April 9, 1946, 77 years old. He was surgeon in the United States Public Health and Marine Hospital Service from 1890 to 1909. From 1909 to 1935 he was professor of preventive medicine and hygiene in Harvard Medical School and from 1922 to 1935 also professor of epidemiology in the Harvard School of Public Health. In 1936 he went to the University of North Carolina, where he directed the new school of public health and was professor of epidemiology. Of Rosenau's researches, the pioneer work by him and Anderson on human anaphylaxis deserves special mention.

Books Received

SYNOPSIS OF PATHOLOGY: By W. A. D. Anderson, M.A., M.D., F.A.C.P., professor of pathology and bacteriology, Marquette University School of Medicine; pathologist, St. Joseph's Hospital, Milwaukee. Second edition. Pp. 722, with 327 illustrations and 15 color plates. Price, about $6. St. Louis: C. V. Mosby Company, 1946.

The first edition was published in 1942. Additions and betterments have been made in practically every chapter. There are some acceptable new illustrations. Colored plates 3, 6, 8 and 15 are decidedly below par. Why should not the larynx receive consideration as well as other parts of the respiratory system? In 1943, 1,490 deaths from cancer of the larynx occurred in this country. In the paragraph on epidemic hepatitis (p. 384) nothing is said about the modes of its causation, and in the statement about acute yellow atrophy of the liver (p. 382) no mention is made of its relation to epidemic hepatitis. But in view of the extent of the book it seems remarkably free from shortcomings. It gives a helpful epitomé of pathology as presented in current textbooks. The morphologic aspects, gross and microscopic, are well described and illustrated.